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LIFE HISTORY STUDIES ON TREMATODES FROM MISSOURI

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The knowledge of North American trematode life histories is very meager. In fact, although about one hundred species of larval trematodes have been described, only four life histories in the United States have been established experimentally. Magath (1918) introduced *Lissorchis fairporti* into the buffalo fish by feeding chironomid larvae experimentally infested with a xiphidiocercaria. Johnson (1920) described in detail the complete life cycle of *Echinostoma revolutum*, finding the adults in ducks and the cercarial and metacercarial stages in the fresh-water snail, *Physa occidentalis*. Tanabe (1923) infested laboratory mice with a new schistosome blood fluke. And Cahn (1927) has obtained adult trematodes from young fish by the direct feeding of an anchor-shaped furcocercous cercaria. The amphistome life history reported by Cary (1909), in the light of the critical analysis by Cort (1915:24), cannot be accepted. The knowledge of European and Asiatic trematode life histories is more extensive. Mathias (1925) lists 21 life histories established outside of North America, and there are others at least partially known.

The material for this study was obtained mainly from Ramona Lake, a small artificial body of water near St. Louis, Missouri. The lake, however, has been in existence over 30 years and is well populated with two species of snails, *Planorbis trivolvis* and *Physa integra*. A wide variety of possible vertebrate hosts are known to live in or frequent the lake. In the course of a two-year survey, a total of 14 different species of larval trematodes were found in the snails of Ramona Lake, and clues were discovered which led to the establishment of two new life histories and the determination of the metacercarial stage of a holostome larvae, *Cercaria hamata* Miller 1923. The new life histories established are those of *Echinoparyphium flexum* (Linton 1892), and *Plagiorochis ameiurensis* sp. nov. from the intestine of the catfish, *Ameiurus natalis*. In addition, *Cercaria ramonae* sp. nov. from *Physa integra* was found to encyst in tadpoles and subsequently develop in snakes into young worms which most probably belong to the genus Renifer. Also, ova from specimens of *Renifer kansensis* Crow 1913, *Dasymetra conferta*

Nicoll 1911, and *Pneumatophilus variabilis* (Leidy) Odhner 1910, obtained from snakes in the vicinity of St. Louis, were used to infest laboratory bred specimens of *Physa integra* and the cercariae of these three forms were obtained. The cercariae encysted in tadpoles, but the entire life cycle was not completed.

This investigation was carried out in the Zoological Laboratory of Washington University from October, 1925, to October, 1927. The writer wishes to thank Dr. Harry M. Miller, Jr., under whose direction the study was conducted, for many helpful suggestions and criticisms. The writer is indebted to Mr. Bryant Walker of Detroit, Michigan, for the identification of the snail hosts.

LIFE HISTORY OF *ECHINOPARYPHIUM FLEXUM* (LINTON 1892)

In the fall of 1926 cysts of an echinostome cercaria were found in large numbers in *Planorbis trivolvis* from Ramona Lake, the infestation in some collections being as high as 60% and individual snails containing as many as several hundred cysts. By experimentally feeding the cysts to young chicks, adult worms, identified as *Echinoparyphium flexum* (Linton 1892), were obtained within a week. Ova from these worms were put into an aquarium with some young laboratory bred snails, *Physa integra*, and at the end of nine weeks, 6 of the 8 surviving snails were infested with an echinostome cercaria found on several occasions in the *Physa integra* of Ramona Lake. Some of the cercariae had emerged and penetrated the snails to encyst. These cysts were identical with those fed at the beginning of the experiment.

The cercaria of *E. flexum* (Fig. 1) resembles other described echinostome cercariae in all general features. The collar spines are in two rows, those in the aboral row being slightly larger than those in the oral row. The four spines on the inside of the ventral shoulders are somewhat larger than the other spines. An exact count of the spines was not possible because of crowding and poor visibility, but more than 40 were usually distinguished. The complete number, 45, counted in encysted worms and adults, is probably also present in the cercaria. The body posterior to the pharynx is crowded with small granular cystogenous gland cells with clear nuclei. Two sets of 4 duct openings are present on the dorsal lip of the mouth, but connections with gland cells were indistinguishable. The main excretory tubule is filled with numerous concretions from the ventral sucker to the pharynx; anterior to the pharynx it loops back and at the posterior end of the ventral sucker receives anterior and posterior collecting tubules. The flame cell pattern was not determined. The excretory tubule in the tail forks one-eighth the length of the tail into branches which open laterally to the outside. The cercariae develop in rediae averaging about 1.0 mm. in length, with a pharynx 0.05 mm. in diameter. In mature rediae the gut extends one-

fifth the length of the body, the locomotor appendages are in the posterior third of the body and a birth pore is present posterior to the collar. Measurements of the cercaria are included in Table 1 in comparison with those for more mature stages in the life cycle.

The pattern of the main ducts of the excretory system of the cercaria of *E. flexum* agrees with that described by Sewell (1922:112) for his Echinatoides and Coronata Groups, but does not agree with the configuration for *C. indica* XXIII, a larva which he ascribes to the genus Echinoparyphium on the basis of the size difference in the collar spines of the oral and aboral rows. In this form he describes no division in the secondary excretory tubule but figures it continuing from the oral sucker to the posterior end of the body and back to the oral sucker unbranched. He hypothecates (1922:121) a branch at the level of the bladder which he could not distinguish. If the cercariae of Echinoparyphium have similar excretory systems, this branch should arise from the secondary tubule immediately posterior to the ventral sucker, but if this be true *C. indica* XXIII cannot remain in his Echinata Group without modification of the group character of the excretory system.

Cysts of *E. flexum* have been found in the mantle cavity of both *Planorbis trivolvis* and *Physa integra* from Ramona Lake, but the percentage of infestation and number of cysts per individual is much greater in the former than in the latter. The cercariae have encysted in laboratory bred snails of both genera. The spherical cysts range from 0.160 to 0.185 mm. in outside diameter, but average about 0.165 mm. The cyst wall consists of two transparent layers, the inner of which is one-half the thickness of the outer. Together they are 7μ in thickness. Concretions are clearly visible in the bladder and main excretory tubules. The spines are clearly visible and are 45 in number, 25 dorsal and 10 on each ventral shoulder.

Linton (1892) described an echinostome from the black scoter in Yellowstone Park, naming it *Distomum flexum;* Dietz (1909; 1910) incorporated this species in his new genus Echinoparyphium. It closely resembles two European species, *E. bacculus* Diesing and *E. recurvatum* von Linstow, both of which also have 45 spines. Since Linton described the reproductive system only in a general way, additional observations are recorded here. The cirrus pouch (Fig. 2) is large and lies dorsal to and on the left side of the ventral sucker. It contains a large seminal vesicle, a prostate gland, and a coiled cirrus which, when protruded, measures 0.5 mm. in length. The vitelline ducts unite just anterior to the testes, but no vitelline reservoir or definite Mehlis gland was distinguished. A large seminal receptacle is located posterior to the ovary and Laurer's canal is present. The uterus extends anteriorly in simple coils to the ventral sucker; it contains from 7 to 15 ova, usually 11. The

vagina is dorsal, slightly to the left of the ventral sucker and opens through the genital pore.

Linton makes no mention of the excretory system. In the present study, this system was observed in immature specimens, 24 and 48 hours old, and in adults. Just posterior to the testes, the bladder receives two large vessels from the anterior part of the body. Between the pharynx and the ventral sucker, there are numerous branched outpocketings from the walls of these vessels which gives them a tree-like appearance. (Text fig. A). This condition was not due to cover glass pressure because it was also observed in immature specimens which were uncovered; this part of the excretory system was not distinguished in adults. Anterior to the pharynx the main tubule loops back on itself and posterior to the ventral sucker receives anterior and posterior collecting tubules. The posterior collecting tubule at the level of the bladder receives 3 tubules

TABLE 1.—*Size Comparison of Stages in Life Cycle of Echinoparyphium flexum*

	Cerc.	Cyst	24 Hr.	48 Hr.	4 Day	Adult
Body Length	0.390	—	0.640	0.900	1.5	2.4
Body Width	0.185	—	0.150	0.210	0.34	0.5
Oral Sucker	0.044	0.048	0.047	0.054	0.078	0.1
Pharynx	0.032×	—	0.043×	0.050×	0.076×	0.079×
	0.018	—	0.021	0.025	0.038	0.047
Ventral Sucker	0.068	0.062	0.088	0.110	0.216	0.30
Dorsal Spine	0.013	0.014	0.030×	0.037×	0.047×	0.052
Aboral Row	× —	× —	0.005	0.006	0.008	× —
Dorsal Spine	0.011	0.012	0.023×	0.028×	0.036×	0.043
Oral Row	× —	× —	0.004	0.005	0.007	× —
Ventral	0.014	0.021	0.039×	0.042×	0.060×	0.065
Shoulder Spine	× —	× —	0.008	0.008	0.010	× —

All measurements are in mm. from living specimens slightly flattened under a cover-glass, with the exception of those for the oral sucker and pharynx of the adult, which are from preserved specimens.

which drain the posterior end of the body. Tufts of cilia are present in the posterior collecting tubules, and in the secondary tubule to a region anterior to the ventral sucker. They beat rapidly in a living specimen flattened under a cover glass. During growth there is a rapid multiplication of flame cells, and in a specimen 4 days old as many as 6 flame cells were observed to drain into a single collecting tubule.

About 200 cysts dissected free from an infested *Planorbis trivolvis* were fed to a chick 2 weeks old. All chicks used in the feeding experiments were laboratory bred, being kept on a board floor and fed only a balanced ration of prepared chick feed. On the eighth day after feeding about 30 large brownish-colored ova were recovered from the feces, but when the chick was killed 4 days later, no worms were found. They may have been overlooked for the intestine was crowded with food. Three more chicks, 4 weeks old, were each fed several hundred cysts, and on the seventh day, when the first fecal examination was made, ova were found in the feces of 2 of the chicks. Seven and 12 adult worms, respectively, were found in the upper fourth of the intestines of these

birds. The third chick was again fed several hundred cysts, but no ova were ever recovered from the feces.

These feeding experiments were repeated on 3 chicks, 16 days old, several hundred cysts being fed to each bird. One chick was killed after 24 hours and 15 young worms were found in the upper fourth of the intestine. At this stage there was no evidence of the development of the reproductive system. Twelve worms were found in the second chick, killed after 48 hours. Two testes about 0.090 mm. in diameter were present anterior to the bladder, but the ovary could only be distinguished as a mass of cells anterior to the testes on the right side. Vitellaria were not present. The third chick was killed 4 days after infestation and 12 worms were found. The cirrus pouch was completely formed, and in one specimen in which the cirrus was protruded, motile sperm were observed to be discharged. The ovary and seminal receptacle were completely formed and vitellaria were present. Although developing ova were distinguished in the ovary, no mature ova were present in the uterus. In another chick fed cysts, ova were recovered from the feces on the fifth day after feeding and were found for 18 days after infestation; no further fecal examinations were made.

Attempts to hatch ova isolated from the feces were unsuccessful. Finally, some feces known to contain ova were placed in an aquarium with 15 small laboratory bred snails (*Physa integra*). After 9 weeks, the 8 snails still alive were killed, and in 6 of the 8, rediae were present, some of which contained mature cercariae identical with the echinostome larva found on several occasions in *Physa integra* from Ramona Lake. Some of the cercariae apparently had emerged and then penetrated the snails to encyst, for 7 of the snails contained from 5 to 50 cysts. These cysts were identical in size, number of collar spines, and general appearance with those fed at the beginning of the experiment.

The life cycle of *Echinoparyphium flexum* is very similar to those described by Johnson (1920) for *Echinostoma revolutum* and by Mathias (1925) for *Hypodaerum conoideum*. The development of the adults is quite rapid, sexual maturity being reached in 5 days, as compared with 8 days for *H. conoideum* and 4 weeks for *E. revolutum*. It is to be noted that although several hundred cysts were fed to each experimental animal, the number of worms found never exceeded 15. The occurrence of such a small number of worms may be explained by the fact that they were developing in an unnatural host. A single attempt to raise the worms in a domestic duck was unsuccessful.

LIFE HISTORY OF *PLAGIORCHIS AMEIURENSIS* SP. NOV.

An examination of some small crayfish collected at Ramona Lake in January, 1927, disclosed the presence of several cysts which were identified as those of a xiphidiocercaria which had been found on

numerous occasions in *Planorbis trivolvis*. Since the stylet was present in the cyst, positive identification was possible. Other crayfish known to contain these cysts were fed to catfish (*Ameiurus natalis*) from the State Fish Hatchery. After three weeks, mature trematodes were found in the intestine and were identified as *Plagiorchis ameiurensis* sp. nov. An identical fluke had been found in the intestine of 4 of 6 young catfish collected at Ramona Lake, in numbers ranging from 1 to 13.

The cercaria of *Plagiorchis ameiurensis* (Fig. 3) is small, varying in length from 0.160 to 0.190 mm., and in width from 0.068 to 0.085 mm. The entire body is covered with small backwardly directed spines completely embedded in the thick cuticula; the spines are more numerous anteriorly. The tail, which is about three-fourths the length of the body, is also finely spined; it is attached to the body ventrally, and at the point of attachment there are lateral pockets which contain short bristles. The stylet is 20 by 5μ in size, and has abrupt shoulders about one-third its length from the point (Fig. 4).

The oral sucker measures 0.044 mm. in diameter and the ventral sucker is somewhat smaller, 0.035 mm. in diameter; the pharynx is 0.013 by 0.015 mm. in size. The ceca are narrow and may be traced only with difficulty to the posterior end of the body. Lateral and anterior to the ventral sucker on each side of the body there are 4 glands which contain clear nuclei. The two posterior glands are somewhat lobulated and lie almost one above the other; they are finely granular and take an acid stain with neutral red. The two anterior glands are coarsely granular and do not stain. Ducts from these glands pass forward to empty at the tip of the stylet. The ducts from the two anterior glands are unstained and wider than the darkly staining ducts from the two posterior glands. The excretory vesicle is "T"-shaped and the sides of the "T" receive the coiled main excretory tubules, which, posterior to the ventral sucker, receive anterior and posterior collecting tubules. Both of these receive 3 accessory collecting tubules which divide into capillaries, but these could not be traced to flame cells. The cercariae develop in typical elongate sporocysts, on the average 1.0 by 0.15 mm. in size. The cercaria of *P. ameiurensis* belongs to the microcotylous group of xiphidiocercariae but cannot be identified with any of the described species.

The cysts have been found in young crayfish, usually in the muscles between the thorax and abdomen and those at the base of the thoracic appendages, but they are also found in the distal joints of the legs and even in the basal parts of the antennae. In a natural infestation not more than 7 cysts have been found, but as many as 100 to 150 occur in experimentally infested animals. There is a great variation in the size of the cyst, which seems to grow after it is formed. The measurements range from 0.115 by 0.093 mm. for a cyst 6 hours old to 0.184

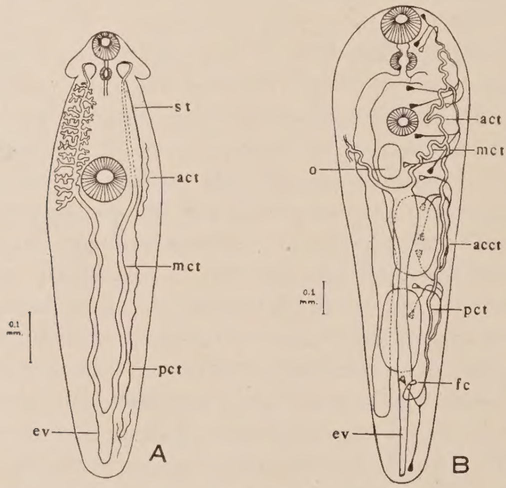
by 0.160 mm. for a cyst of maximum size; the average size of 12 specimens was 0.147 by 0.121 mm. The excretory vesicle of the encysted worm is always filled with a dark mass of concretions. The most radical difference from the cercarial stage to be noted in the metacercariae dissected from mature cysts is the increased conpicuousness of the digestive tract. The prepharynx is wide and extensible; the esophagus is short and forks into wide ceca which appear clear and extend to the end of the body. The testes can be seen developing on either side of the elongated excretory vesicle, the left one being diagonally anterior to the right. Cysts have also been obtained from dragon fly larvae which had been exposed to the cercariae, but no natural infestation of insect larvae has been noted. Several times cysts have been found in the mantle cavity of snails from Ramona Lake, but this location is probably abnormal.

Plagiorchis corti Lamont 1921, from *Schilbeodes gyrinus* is the only species of Plagiorchis previously reported from fishes. Although the present species, *P. ameiurensis* (Fig. 5) is found in a closely related genus, Ameiurus, and is very similar to *P. corti,* it is described as a new species because of some structural differences and size discrepancies. *P. corti* measures 1.0 by 0.3 mm. while *P. ameiurensis* is 1.50 by 0.37 mm. Since Lamont gives no other measurements for her species, no further size comparisons are possible. In the figure of *P. corti* the coils of the uterus do not extend posterior to the ceca, but in *P. ameiurensis* the loop of the uterus is definitely posterior to the ceca which do not quite reach the end of the body. Lamont (1921) says that the ovary of *P. corti* is "slightly posterior and to the left of the ventral sucker," but in the figure it is shown on the right side opposite the cirrus pouch which is dorsal and to the left of the ventral sucker. This condition is contrary to the characteristic of the genus as given by her, "Ovary spherical, at inner end of cirrus sac." In *P. ameiurensis* both the cirrus pouch and ovary are on the right side, the ovary being directly posterior to the end of the cirrus pouch. A further difference between the two species is that the ceca of all specimens of *P. ameiurensis* are of wide diameter, whereas in *P. corti* they are figured quite narrow.

The genus Plagiorchis contains many species some of which have been reported from amphibians, reptiles and mammals, but most of which are from birds. The present species possesses all of the typical characteristics of the genus except that the vitellaria reach only to the posterior testis instead of to the end of the body. Also, spines, although present over the entire body, are very scarce posterior to the testes. The following measurements are the averages from 5 preserved specimens: body, 1.50 by 0.37 mm.; oral sucker, 0.12 mm. in diameter; ventral sucker, 0.086 mm. in diameter; pharynx, 0.058 mm. in diameter; ovary,

0.137 mm. in diameter; ova, 0.034 by 0.020 mm.; anterior testis, 0.176 mm. in diameter, and posterior testis, 0.194 mm. in diameter.

The cirrus pouch contains a large seminal vesicle, a prostate gland, and a coiled muscular cirrus. The vasa efferentia unite on the mid-line anterior to the ovary to form a short vas deferens. The oviduct leaves the ovary on the median side, and curves posteriorly a short distance, Laurer's canal branching off at this point. The oviduct then turns anteriorly to an indistinct Mehlis gland posterior to the ventral sucker and receives the vitelline ducts which have united to form a small vitelline reservoir. The uterus coils ventral to the ovary, continuing between the testes to the posterior end of the body where it forms a single loop to pass anteriorly, either between the testes or ventral to the



Text Figure.—*A*. Free hand drawing to show the excretory system of a living specimen of *Echinoparyphium flexum* 48 hours after feeding the cysts. *B*. Diagram of the excretory system of an adult *Plagiorchis ameiurensis*. *act*, anterior collecting tubule; *acct*, accessory collecting tubule; *c*, capillary; *ev*, excretory vesicle; *fc*, flame cell; *mct*, main collecting tubule; *o*, ovary; *pct*, posterior collecting tubule; *st*, secondary tubule.

left testis. It continues as a thin-walled vagina and passes on the left side of the ventral sucker to open at the genital pore, in the median line immediately anterior to the sucker. The uterus in mature individuals is crowded with an immense number of ova. The vitellaria appear as several rows of follicles along the sides of the body from the region of the pharynx to the anterior margin of the posterior testis.

The excretory system studied in mature living specimens (Text fig. B) was found to be of the "2 × 6 × 3" type, and is almost identical with that described by Cort (1919b) for *Margeana californiensis*. The chief difference between the two is that in *P. ameiurensis* the paired

flame cells of each group are regularly on the dorsal side of the body, and the unpaired capillary on the ventral side, whereas in *M. californiensis* the paired flame cells, although always on the same side of the body, may be either dorsal or ventral.

In preliminary experiments young trematodes were found in the intestines of three catfish known to have eaten parts of crayfish containing cysts. Thirteen catfish (*Ameiurus natalis*) 3 to 6 inches in length, were procured from the Missouri State Fish Hatchery for a controlled feeding experiment. Five of the fish were killed at once and no trematodes were found. Each of the remaining 8 fish was fed a crayfish from Ramona Lake naturally infested with cysts, about 1 to 5 cysts per animal. One of the fish (1) killed after 12 days contained one very young trematode with the testes and cirrus pouch formed but the female genital system undeveloped. Another (2) killed on the 18th day after feeding, harbored 3 trematodes of a more advanced stage of development with the female reproductive system fully formed and about 30 ova present in the uterus. After 3 weeks, 3 more fish (3, 4, 5) were killed, two of which contained no worms, but the third contained one trematode nearly of adult size.

Eighteen days after the original feeding each of two of the catfish was fed portions of a crayfish, which had been exposed to cercariae nearly 3 weeks previous. The parts of the two crayfish were examined and estimated to contain 50 and 100 cysts respectively. One week later the two catfish were killed. The fish fed 50 cysts contained 25 very young worms, all at the same stage of development. The other fish which ate 100 cysts harbored 66 very young worms and two practically adult worms, which probably developed from the original feeding 25 days previous. The last fish killed later proved negative, so that of the 8 fish originally fed a small number of cysts, 5 developed trematodes which were recovered in an immature stage. Two of the fish were fed a large number of cysts and developed a heavy infestation, 25 and 66 worms respectively. In a later experiment cysts from an experimentally infested dragon fly larva were fed to a very young catfish from a lot 6 of which had been examined and were found to be free from trematodes. The fish was killed after 6 days and 18 young worms, all at the same stage of development, were recovered from the intestine. The sum of this evidence is considered sufficient to establish the life history.

Presumptions based on structural similarity have been made (Faust, 1918:104; Cort, 1919a:295; Sewell, 1922:232) that xiphidiocercariae are the larval forms of Plagiorchiidae and possibly of closely related forms. *Haplometra cylindracea* and *Opisthioglyphe ranae* are known to develop from xiphidiocercariae (Lühe, 1909). The life history reported by Magath (1918) identifies a xiphidiocercaria as the larva of *Lissorchis fairporti,* a species of a new sub-family related to the Plagiorchiidae.

The present work definitely establishes a microcotylous xiphidiocercaria as the larva of a species of Plagiorchis.

The development of *P. ameiurensis* in the fish is not nearly as rapid as the development of *Echinoparyphium flexum* in the chick. At the end of a week, the testes of the young worm are formed and the cirrus pouch is beginning to develop, but there are no definite structures of the female genital system present. This condition is exactly opposite to that in *Lissorchis fairporti* in which the ovary is the first part of the reproductive system to develop. After 12 days the male system of *P. ameiurensis* is fully formed but the female system is just beginning to develop. In 18 days the worm is sexually mature; the vitellaria are formed and some ova are present in the uterus. After 25 days the number of ova has increased, but the enormous number present in the adult uterus is probably not reached for another week or two. The above schedule of development is based on the feeding experiments which were performed in March when the temperature of the aquarium was about 18°C. In the later experiment performed in July when the water temperature was about 27°C., the worms recovered 6 days after feeding were at about the same stage of development as the worms recovered 12 days after feeding in the earlier experiments, indicating that development is much more rapid at the higher temperature. An attempt made to hatch ova in the laboratory and to infest snails experimentally with the miracidia was unsuccessful.

METACERCARIA OF *CERCARIA HAMATA* MILLER 1923

In several preliminary experiments it was noticed that small sunfish (*Eupomotis gibbosus*), left in a small aquarium with snails giving off *Cercaria hamata* Miller 1923, a pharyngeal longifurcate "mono stome" cercaria, died within a few days, while other fish in a control aquarium continued to live. In October, 1926, 4 sunfish were exposed to a large number of *C. hamata* in a small aquarium for short periods of time, 5 to 20 minutes. The fish were removed to a larger aquarium, and after 4 weeks, darkly pigmented cysts, about 0.51 by 0.46 mm., were observed in the fins and beneath the skin of the fish. Each cyst, when dissected, was found to contain a young, undifferentiated holostome. In November the experiments were repeated on 24 small sunfish, but since the supply of cercariae was very limited, the infestation was not satisfactory, and at the end of 6 weeks, of the 17 fish still alive, only 9 contained cysts. The greater length of time required for the cysts to develop in this second experiment may be explained by the fact that the tap water run into the aquarium daily became colder, the temperature falling as low as 14°C.

In June, 1927, a lot of 18 small sunfish (*Eupomotis gibbosus*) was procured from the State Fish Hatchery, and 14 of them were exposed

to a large number of *C. hamata* in a small aquarium for periods of time varying from 15 minutes to 24 hours. Four fish were kept as controls. In the course of the experiment, 7 of the exposed fish died: 4 of these were examined and found to contain numerous penetrated cercariae. Three of the exposed fish killed at intervals after the exposure were all infested, and of the 4 remaining fish, all showed cysts in the fins and beneath the skin between 2 and 3 weeks after the infestation. None of the 4 control fish contained cysts. The temperature of the water in the aquarium was about 25°C.

Numerous penetrated cercariae were found in a fish killed 48 hours after exposure. The worms were present all over the body, greatest preference being shown for the muscles at the base of the fins. The young worm is only slightly larger than the body of the cercaria; it contains no developed structures but is filled with opaque undifferentiated cells. The outline of the oral sucker is definite but the cells composing it are undifferentiated. The worms are free in the tissue and are capable of slow movement. At the end of 5 days, the worms are contained in a thin transparent cyst, probably secreted by the tissues of the fish. They are free within the cavity of the cyst, and aside from an increase in size, no signs of further development are visible. Two weeks after penetration, the body of the metacercaria is now much larger, 0.38 by 0.26 mm., and has developed a tail-like appendage on the posterior dorsal side which is 0.12 by 0.13 mm. At the posterior end of the body proper is a round structure 0.075 mm. in diameter, which is apparently the beginning of the adhesive organ. Just anterior to this structure is the round outline of the developing ventral sucker, 0.033 mm. in diameter. The pharynx lies close behind the oral sucker and is followed by a short esophagus which almost immediately branches into 2 ceca which could not be traced posteriorly. Small globules, probably excretory in nature, are distributed beneath the surface of the body but no definite excretory tubules were distinguished. The cyst containing the worm is 0.41 by 0.37 mm. in size and its walls contain a small amount of scattered black pigment.

Three weeks after the penetration of the cercariae, the cysts have grown to be 0.55 by 0.38 mm. in size. The worms are still free within the cysts and are considerably larger, the body measuring 0.44 by 0.32 mm. and the posterior appendage 0.25 by 0.10 mm. The two suckers, the pharynx, and adhesive organ appear definitely but the cells of the rest of the body are undifferentiated. The digestive ceca could be traced to the end of the posterior appendage. Numerous concretions are present in the excretory tubules but the exact pattern of the system was not traced. In an unflattened condition, the posterior edge of the body curls ventrally to give a cup-shaped appearance to the body, from which the adhesive organ protrudes ventrally. The cyst

wall is now covered with black pigment so that in the living fish, the cysts are easily visible to the naked eye.

The final stage of development of the metacercaria is reached after 4 weeks. The worm is no longer free within the cyst, but is enclosed in a thin transparent ovoidal capsule which is much smaller than the cavity of the cyst, measuring 0.38 by 0.22 mm. (Fig. 6). This capsule apparently is secreted by the worm. The structures of the metacercaria appear sharply differentiated, and the worms exhibit considerable movement inside the capsule. The metacercaria apparently has reached its ultimate development for no further changes were noted in cysts dissected one month later. The posterior appendage is folded up ventrally against the body and fills the largest end of the cyst; the suckers, pharynx, and adhesive organ are in the other end of the cyst, usually in distorted positions. The excretory tubules are packed with concretions and extend throughout the body in a complicated pattern.*

An attempt was made to discover whether fish previously exposed to *C. hamata* would be more resistant to a second infestation than fish free from cysts. Eighteen sunfish from a lot of 32 were exposed for 30 minutes to an immense number of *C. hamata* in a small aquarium; the remainder were kept as controls. This exposure proved to be sufficient to heavily infest all of the exposed fish. One, 2, 3, and 4 weeks after this infestation, 2 fish from this lot were exposed for 2 hours to a large number of cercariae, and 2 control fish were similarly treated. After 48 hours when the fish were killed, in every trial, the previously exposed fish contained approximately the same number of penetrated cercariae from the second exposure as did the control fish. In a further test, 3 weeks after the original infestation, 6 of the previously exposed fish and 6 control fish were left overnight in an aquarium with a large number of cercariae, an exposure thought to be sufficient to cause the death of the fish. Within 5 days all of the fish had died, the previously exposed fish dying at about the same rate as the control fish. These rough experiments seem to indicate that even a very heavy infestation of the fish with the metacercariae does not make the fish more resistant to a second infestation.

C. hamata is the first cercaria from the United States to be experimentally identified as a larval holostome. Through the work of Lutz (1921), Ruszkowski (1922), Szidat (1924; 1925), and Mathias (1925), some furcocercous distome cercariae have been identified as holostome larvae, but heretofore there have been no clues connecting the "pharyn-

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* Hughes has published (1927) a very detailed description of a holostome metacercaria which he assigns to a new larval genus, Neascus. The metacercaria of *C. hamata*, which is very similar to the form described by Hughes, would belong in this genus, and the work of the present study indicates the type of cercaria to be identified with the group.

geal longifurcate monostome" larvae with any group of adult trematodes. Miller (1926:72) recognizes that the absence of a ventral sucker in this group is probably not significant, and the present study in identifying the metacercarial stage of *C. hamata* as a larval holostome bears out his conclusion.

Szidat (1925) discovered that the pharyngeal longifurcate distome, Cercaria C, penetrates various fresh-water fish and develops in the eye into a larval hemistome, *Diplostomum volvens,* which is known to develop into *Hemistomum spathaceum,* parasitic in the intestine of numerous water birds. He observed that fish exposed to a thick cloud of Cercaria C die within a few hours, bleeding both internally and externally. *C. hamata* has not had such a severe effect on sunfish even when penetrating in large numbers; the fish did not die for several days, and no hemorrhage was ever noticed. Other references to the penetration of fish by furcocercous cercariae are reviewed by Miller (1926:79-80).

Diplostomum cuticola is described by von Nordman (1832) from darkly pigmented cysts occurring in the eyes, skin, and fins of young minnows. This species has been reported from numerous fresh-water fish in North America (Ward and Whipple, 1918:411), but the larva reported from the sunfish by Linton (1898) as *D. cuticola* cannot be identified with the metacercaria of *C. hamata.* Agersborg (1926) reported *Diplostomum van cleavi* as occurring in the body cavity of minnows from Urbana, Illinois. Sometimes these worms were in a capsule-like cyst, but usually they were free in the body cavity. Many other larval trematodes have been reported from fishes but often they cannot be identified as belonging to the Strigeidae, and usually they are localized in some particular region of the body, the eye being a preferred location. The cases of holostome larvae occurring in the eye of fishes are reviewed by La Rue, Butler, and Berkhout (1926).

Small sunfish from Ramona Lake, in which about 5% of the *Planorbis trivolvis* are infested with *C. hamata* are almost invariably naturally infested with large numbers of the cysts, one small specimen containing an estimated number of over 5,000. The cysts have also been noted in small numbers in bass from the lake, but they have never been found in tadpoles or catfish. In attempts to develop adults, cysts have been fed to white rats, chicks, young domestic ducks, a sparrow hawk, snakes, and turtles, but no worms have been recovered.

CERCARIAE BELONGING TO THE SUBFAMILY RENIFERINAE PRATT

The subfamily Reniferinae Pratt is a well-defined group of Trematodes within the family Plagiorchiidae parasitic in the mouth, air passages, lungs, esophagus, and stomach of snakes. The exact classification of this group is in dispute; recently Baer (1924) raised it to the rank of a family Reinferidae, distinct from Plagiorchiidae and excluded

certain genera which Odhner (1910) had included in the group, notably Pneumatophilus Odhner and Leptophallus Lühe. Baer's classification is based on the Y-shape of the excretory vesicle and particularly the lateral branches from the main stem. This latter character, however, has not been observed in all species which should fall into this group (Sumwalt, 1926). In this paper Reniferinae will be treated as a subfamily of the Plagiorchiidae with the group characters as defined by Pratt (1903).

Nothing definite is known regarding the life cycle of any members of the Reniferinae. In this investigation, cercariae have been developed in snails from the ova of representatives of three genera—Renifer, Dasymetra, and Pneumatophilus, all members of this subfamily. In addition *Cercaria ramonae* sp. nov. has been found experimentally to encyst in tadpoles, the cysts have been fed to snakes, and immature worms recovered from the snakes which almost certainly belong to the genus Renifer.

Cercaria ramonae sp. nov.

On numerous occasions a cercaria has been found in the *Physa integra* and *Physa anatina* from several localities near St. Louis which very closely resembles *C. brevicaeca* Cort 1914. This cercaria, which will be called *Cercaria ramonae* sp. nov. (Fig. 7), differs from *C. brevicaeca* chiefly because it is larger, being 0.500 by 0.175 mm. as compared with 0.300 by 0.140 mm. The oral sucker is 0.073 mm. in diameter and the ventral sucker is somewhat larger, 0.086 mm. in diameter, which measurements are practically the same as those for the suckers of *C. brevicaeca.* The stylet, however, is larger 23μ in length as compared with 18μ, and is slightly different in shape (Fig. 8). *Cercaria ramonae* possesses all of the body characters of *C. brevicaeca,* short ceca not extending posterior to the ventral sucker, about 10 pairs of stylet glands in which clear nuclei could easily be distinguished, and most notably an excretory vesicle with lateral arms nearly encircling the ventral sucker. When the living animal is contracted and the vesicle distended, the two arms often appear to join anterior to the ventral sucker. Cort gives no further description of the excretory system of *C. brevicaeca.* Observations upon the excretory system of *Cercaria ramonae* were not complete, but it was definitely determined that the main collecting tubules empty into the lateral arms of the excretory vesicle just posterior to the ventral sucker, and that there are 3 anterior and 3 posterior accessory collecting tubules on each side of the body. The two most anterior of the posterior accessory tubules empty very close together into the anterior part of the posterior collecting tubule which is so greatly coiled that it obscures the course of the capillaries. Flame cells were located in other groups, however, and all observations indicate that the system is of the "$2 \times 6 \times 3$" type.

Cercaria ramonae readily encysts in tadpoles and young catfish exposed to the cercariae, but does not encyst in dragon fly larvae. Cysts have been found as a natural infestation of tadpoles, young frogs, and catfish. The cysts vary considerably in size, but average 0.26 by 0.20 mm. In the tadpoles they are present in the soft tissues all over the body, but in the catfish they are mainly in the tissue around the pharynx and in the mesenteries of the upper intestine. The worm inside the cyst has changed very little from the cercaria, except that tail and stylet are no longer present, and the bladder is filled with numerous concretions which give it a very dark appearance in contrast to the body of the metacercaria. In nearly all specimens the lateral arms of the excretory vesicle seem to join one another anterior to the ventral sucker.

Tadpoles experimentally infested with *Cercaria ramonae* were fed to 3 specimens of the common water snake, *Natrix sipedon*. One snake was killed after 3 days, another after 2 weeks, and the third 6 weeks after feeding the cysts, and 17, 39, and 3 young worms respectively were found in the lower part of the esophagus. These worms are only slightly larger than the encysted worms and are not much more advanced in development (Fig. 9). The excretory vesicle is still filled with dark concretions. Stained specimens show two testes in the same transverse plane posterior to the ventral sucker, and an ovary dorsal to the ventral sucker on the right side of the body from which a uterus extends diagonally to the left margin of the body just posterior to the pharynx, indicating the position of the genital pore. The ceca still do not extend posterior to the ventral sucker, but judging from the position of the genital pore, the young worms most probably belong to the genus Renifer.

Eight young worms almost identical with these experimental worms were found in the esophagus of a young specimen of *N. sipedon* from Ramona Lake, a locality known to contain *Cercaria ramonae* in abundance. This finding would seem to indicate that the experimental worms were immature not because they were in an unnatural host but because they had not had sufficient time to develop. The length of time required for the worms to become fully grown is problematical; if at the end of 6 weeks, they are not nearly sexually mature, it may be a year or more before they are full grown. It should be noted that a young turtle, *Chelydra serpentina*, was fed a tadpole infested with cysts of *Cercaria ramonae*, and when killed after 6 weeks, 4 young worms were found in the esophagus identical with those which had been found in the snakes.

CERCARIA OF *RENIFER KANSENSIS* CROW 1913

A black snake, *Coluber constrictor*, from southeastern Missouri contained two worms in the upper esophagus which were identified as *Renifer kansensis* Crow 1913. These worms agreed with *R. kansensis*

in size and all bodily characters except the length of the ceca. Crow describes the ceca of *R. kansensis* as reaching the middle of the body and figures them ending a short distance anterior to the testes. In one of the present specimens the ceca extended slightly posterior to the testes; in the other specimen the left cecum extended to the middle of the testis, while the right cecum did not quite reach the testis. In view of the fact that the exact extent of the ceca does not seem to be a fixed character, the difference between the worms of the present study and *R. kansensis* is not considered sufficient to warrant the establishment of a new species.

Ova from these two specimens of *Renifer kansensis* were placed in an aquarium with some laboratory bred *Physa integra*. At the end of two weeks several snails were killed and found to be infested with sporocysts, but the rest of the snails all died before any cercariae matured. Fortunately another specimen of *Renifer kansensis* was obtained from a king snake, *Lampropeltis getulus,* and ova from the worm were placed in an aquarium with 24 pond snails (*Physa anatina*), and 24 laboratory bred *Physa integra*. After 3 weeks 8 of the *Physa anatina* were still alive; they were isolated in vials and 7 of them gave off mature cercariae. The eighth snail was killed and found to be uninfested. Fifteen of the *P. integra* were alive but none of them gave off cercariae. They were killed and 11 of them contained small oval sporocysts, but in only one of these infestations were there developed cercariae.

The cercaria of *Renifer kansensis* differs from *Cercaria ramonae* only in several minor points. The body is smaller and more rounded, measuring 0.44 by 0.20 mm. The stylet is similar in appearance to that of *Cercaria ramonae* but is smaller, being 19μ in length; the suckers are also proportionately smaller. The lateral arms of the excretory vesicle, although extending anterior to the ventral sucker, do not appear to join one another, and in some specimens apparently contain coarse globular material. The pattern of the excretory system was not determined. The cercaria of *Renifer kansensis* encysted in young catfish which were exposed to the cercariae for 18 hours. The cysts are smaller than those of *Cercaria ramonae,* measuring 0.20 by 0.18 mm. The excretory vesicle contains irregular concretions and is distinctly Y-shaped; the lateral arms do not appear to join one another as in the metacercaria of *Cercaria ramonae*. It was not possible to attempt feeding experiments with these cysts.

CERCARIA OF *DASYMETRA CONFERTA* NICOLL 1911 (FIG. 10)

One specimen of *Natrix sipedon* from the vicinity of St. Louis contained about 50 mature worms in the upper esophagus which were identified as *Dasymetra conferta* Nicoll 1911. The agreement of these

specimens with Nicoll's description was very close. Size measurements were practically identical, and the peculiarly characteristic pigmented appearance of the excretory tubules was very striking. Nicoll, who studied only preserved material, was uncertain as to whether the darkened appearance might not be a post-mortem condition, but this study proves that it may also be seen in living animals, and is caused by the presence of numerous very small concretions all over the excretory system, even in the capillaries. Nicoll also was uncertain about the habitat of the worms, which is now known to be the upper esophagus. The pattern of the excretory system was not described; from a study of living material it was found to be of the "$2 \times 6 \times 3$" type.

Numerous ova from these worms were placed in an aquarium with 24 laboratory bred *Physa integra*. After 4 weeks 12 of the snails were still alive, and 11 of them were infested with a cercaria superficially very similar to *Cercaria ramonae*. Subsequently two additional specimens of *Dasymetra conferta* were obtained from another snake, and the infestation experiments were repeated on 40 laboratory bred specimens of *Physa integra*. After 4 weeks 37 of the snails survived and 13 of them were found to be infested with the same cercaria as in the first experiment. The survival of a larger number of the snails and the lower percentage of infestation in this second experiment may be explained by the fact that fewer ova were placed in the aquarium with the snails.

The body of the cercaria of *D. conferta* is smaller than that of *Cercaria ramonae*, 0.34 by 0.16 mm., but the stylet is exactly the same length, 23μ; the thickening, however, is somewhat more pronounced (Fig. 11). The tail is normally about three-fourths the length of the body; the suckers are the same size, 0.068 mm. in diameter. The pharynx is large, 0.030 mm. in diameter, the esophagus is moderately long, and the ceca extend to the posterior end of the body. Two sets of about 8 coarsely granular glands are present anterior to the ventral sucker, and ducts from them pass forward to empty at the tip of the stylet; no nuclei could be distinguished in the glands. All specimens contain many round fat-like globules of varying size distributed all over the body, and in addition there are numerous clusters of much smaller irregular concretions.

The excretory vesicle possesses lateral arms which extend to the anterior margin of the ventral sucker, but never appear to encircle it. These arms are not as pronouncedly separated from the stem of the excretory vesicle as in *Cercaria ramonae*. The main collecting tubules empty into the lateral arms just posterior to the ventral sucker; the excretory pattern of the cercaria was determined also to be of the "$2 \times 6 \times 3$" type.

The cercaria of *D. conferta* encysted in tadpoles in large numbers. The cysts vary from 0.25 to 0.38 mm. in length and from 0.22 to

0.33 mm. in width, but average about 0.35 by 0.31 mm. The excretory vesicle is filled with dark concretions but is not as prominent as in the metacercaria of *Cercaria ramonae*. The ceca contain granular material and are difficult to trace. With the exception of the suckers and pharynx, the whole body appears undifferentiated; no trace of stylet glands is still present.

CERCARIA OF *PNEUMATOPHILUS VARIABILIS* (LEIDY) ORHNER 1910 (FIG. 12)

A single specimen of *Natrix sipedon* from the vicinity of St. Louis harbored in the trachea, 6 large worms which were identified as *Pneumatophilus variabilis* (Leidy) Odhner 1910. Size measurements of these specimens were somewhat greater than those given in the accepted description of the species by Pratt (1903), but all features of the body agreed very closely.

Ova from these worms were placed in an aquarium with 24 laboratory bred *Physa integra*. In the course of 4 weeks, 6 of the snails were killed, and 3 of them were infested with sporocysts but no developed cercariae were present. At the end of 5 weeks, 4 of the snails were still alive and were isolated in vials. Two of the snails gave off cercariae, similar in bodily structure to *Cercaria ramonae,* and also superficially resembling the cercaria of *D. conferta*. The other two snails were killed and were not infested.

The cercaria of *P. variabilis* is approximately the same size as that of *Renifer kansensis;* the suckers are somewhat larger, the oral sucker measuring 0.056 mm. and the ventral sucker 0.059 mm. in diameter. The stylet is slightly longer, 20μ in length, and has a definite thickening 5μ from the point (Fig. 13). The pharynx is small, 0.028 mm. in diameter, and the prepharynx is only one-half the diameter of the pharynx. The esophagus is moderately long, but the ceca do not extend posterior to the ventral sucker. Anterior to the ventral sucker are two sets of 8 to 10 coarsely granular glands; clear nuclei are present in them but are difficult to distinguish. The excretory vesicle is the same general Y-shape as in *Cercaria ramonae,* but the main stem is shorter and the lateral arms are more definitely separated from each other and the main stem of the vesicle; they extend definitely anterior to the ventral sucker. The flame cell pattern was not distinguished. No second intermediate host has been experimentally determined for this cercaria.

DISCUSSION

The foregoing observations indicate the general characters of the cercariae of the genera, Renifer, Dasymetra, and Pneumatophilus to be as follows:

Body large, about 2½ times as long as broad, entirely covered with small spines, and developing in sporocysts. Tail normally about three-

fourths the length of the body, and not spined. Heavy stylet slightly thickened near point. From 8 to 10 pairs of stylet glands with ducts emptying at tip of stylet. Suckers of equal size in Dasymetra, ventral sucker slightly larger than oral sucker in Renifer and Pneumatophilus. Ceca not extending posterior to the ventral sucker in Renifer and Pneumatophilus, but nearly reaching posterior end of body in Dasymetra. Excretory vesicle Y-shaped with crura extending at least to anterior margin of ventral sucker; main collecting tubules emptying into crura just posterior to ventral sucker. Flame cell pattern in Renifer and Dasymetra of the "$2 \times 6 \times 3$" type.

Measurements of the cercariae given in the foregoing accounts were taken from individuals killed by gentle heat. Measurements of the stylets are for the hardened portion and do not include the basal core.

The Y-shape of the excretory vesicle of *C. brevicaeca* with the lateral arms nearly encircling the ventral sucker, at the time of Cort's description (1915), was unique. The four cercariae in the above account all possess this shape of vesicle, and with the exception of the cercaria of *D. conferta* might possibly be identified with *C. brevicaeca*. All four of the cercariae are very similar in general appearance, behavior, and bodily structure, differing only in minor details, with the exception of the long ceca in the cercaria of *D. conferta*. Positive identification of the cercariae would be very difficult unless the observer had all forms for comparison.

In Renifer and Pneumatophilus apparently, the ceca must grow further posterior with the growth of the worm. In the cercaria the ceca do not extend beyond the ventral sucker, while in adults they reach the posterior end of the testes. An intermediate condition has been observed in specimens of both genera which were not quite sexually mature; in these worms the ceca extended beyond the ventral sucker but ended anterior to the testes. Although the complete life history has not been determined for any of the worms, the most probable cycle seems to be: adults in snakes, cercariae in snails of the genus Physa, and metacercariae in tadpoles and young frogs. The feeding experiments indicate that the development of the worms within the snake is quite slow. The great similarity of the cercariae of the two species of Renifer and *Pneumatophilus variabilis* apparently indicates a fairly close relationship between the two genera and seemingly would not justify Baer (1924) in excluding Pneumatophilus from the group Reniferinae.

SUMMARY OF RESULTS

1. An echinostone, *Echinoparyphium flexum* (Linton 1892) was experimentally developed to maturity in chicks by feeding cysts from *Planorbis trivolvis*. Ova from experimentally obtained adults were used

to infest laboratory bred specimens of *Physa integra* and mature cercariae developed in the snails. These cercariae formed cysts which were identical with those originally fed to the chicks. New observations are added to Linton's description of the adult.

2. A xiphidiocercaria was observed to encyst in crayfish and dragon fly larvae. These cysts fed to the catfish, *Ameiurus natalis,* developed to *Plagiorchis ameiurensis* sp. nov. The excretory pattern in the adult is of the "$2 \times 6 \times 3$" type; incomplete observations indicate that it is probably the same in the cercaria.

3. *Cercaria hamata* Miller 1923, a "pharyngeal longifurcate monostome" cercaria, was found to penetrate sunfish (*Eupomotis gibbosus*) and to develop into a larval holostome. Infestation experiments indicate that the presence of a large number of cysts in the fish does not make the fish more resistant to a second infestation.

4. *Cercaria ramonae* sp. nov., a xiphidiocercaria from *Physa integra,* was observed to encyst in tadpoles and develop in the water snake, *Natrix sipedon,* into young worms which most probably belong to the genus Renifer.

5. Ova from specimens of *Renifer kansensis* Crow 1913, *Dasymetra conferta* Nicoll 1911, and *Pneumomatophilus variabilis* (Leidy) Odhner 1910, obtained from snakes from the vicinity of St. Louis, were used to experimentally infest laboratory bred specimens of *Physa integra* and the cercariae of these three forms were obtained and described. The larvae belong to the xiphidiocercariae and are very similar to each other and *Cercaria ramonae* mentioned above as developing into a species of Renifer. These four cercariae indicate the general characteristics of the larvae of Reniferinae, the most striking of which is the Y-shaped excretory vesicle with the lateral arms extending anterior to the ventral sucker.

6. The excretory pattern of both the adult and the cercaria of *Dasymetra conferta* was determined to be of the "$2 \times 6 \times 3$" type.

7. The similarity of the cercariae of the two species of Renifer with the cercaria of *Pneumatophilus variabilis* apparently does not justify Baer (1924) in excluding Pneumatophilus from the group Reniferinae.

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Explanation of Plate X

The projected scale has a value of 0.1 mm. in all figures except Nos. 4, 8, 11, and 13; in these it has a value of 0.01 mm.

Figures 2, 5, 6, and 9 were drawn with the aid of a camera lucida; all other drawings are free hand

Abbreviations: *gp,* genital pore; *ov,* ovary; *sr,* seminal receptacle; *t,* testis; *ut,* uterus.

Fig. 1.—Dorsal view of the cercaria of *Echinoparyphium flexum.*

Fig. 2.—Ventral view of an adult *Echinoparyphium flexum.*

Fig. 3.—Ventral view of the cercaria of *Plagiorchis ameiurensis.*

Fig. 4.—Stylet of the cercaria of *P. ameiurensis.*

Fig. 5.—Ventral view of an adult *Plagiorchis ameiurensis.*

Fig. 6.—Capsule of the mature metacercaria of *Cercaria hamata* dissected free from the cyst.

Fig. 7.—Ventral view of *Cercaria ramonae,* larva of a species of Renifer.

Fig. 8.—Stylet of *Cercaria ramonae.*

Fig. 9.—Dorsal view of a young specimen of Renifer sp. from esophagus of snake 6 weeks after feeding cysts of *Cercaria ramonae.*

Fig. 10.—Ventral view of the cercaria of *Dasymetra conferta.*

Fig. 11.—Stylet of the cercaria of *D. conferta.*

Fig. 12.—Ventral view of the cercaria of *Pneumatophilus variabilis.*

Fig. 13.—Stylet of the cercaria of *P. variabilis.*

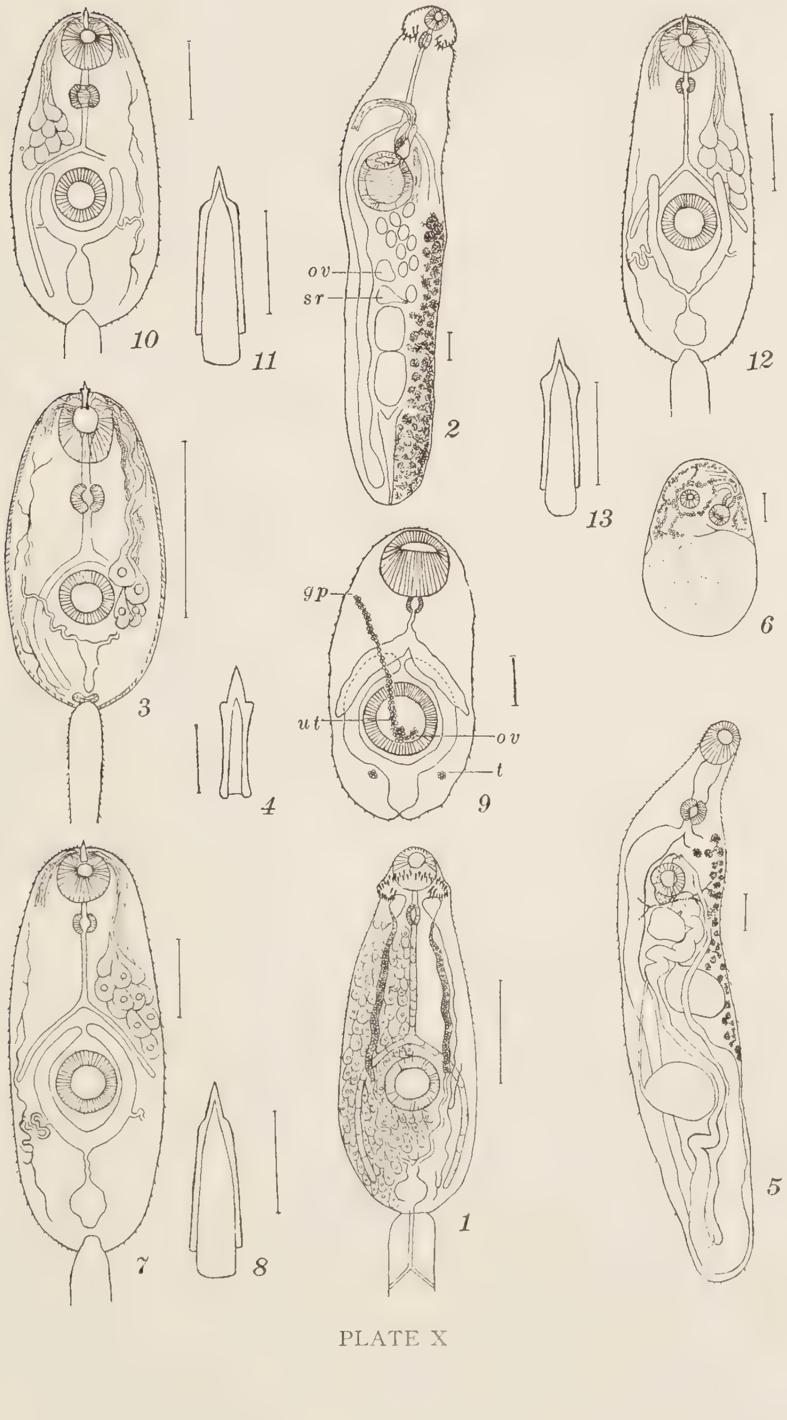

PLATE X

CONTRIBUTIONS TO THE LIFE HISTORY OF *PROTEOCEPHALUS AMBLOPLITIS* (Leidy)*

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Proteocephalus ambloplitis was first described as *Taenia ambloplitis* by Leidy in 1887. In 1900 Benedict identified as this species some material in the Ward collection. Marshall and Gilbert (1905) mentioned it in connection with a study of the food of fishes from lakes in the vicinity of Madison, Wisconsin. La Rue, in his monograph on the Proteocephalidae (1914), brought much material on this species together and determined the synonomy of the forms described by Riggenbach (1896), Linton (1897), and other workers. He carefully redescribes the parasite and lists four new localities where it is found. This work, because of its completeness, renders further study of the adult unnecessary.

Leidy (1887) described what is now known to be a plerocercoid as *Taenia micropteri*. This was found in the intestine of *M. salmoides* (= *nigricans*) from Lake George, N. Y. La Rue (1914) suggested that this larva was probably that of *P. ambloplitis*. Cooper (1915) while making a systematic study of the fresh-water fishes of the Georgian Bay region, noted some plerocercoid larvae in the small-mouth black bass (*M. dolomieu*). By comparison of the adult characters with those of the larva, Cooper showed that the latter was *P. ambloplitis*. He was unable to determine the life cycle of the parasite, but states that "the evidence points to *P. ambloplitis* having at least two intermediate hosts, the first, some unknown species of aquatic arthropod, and the second, either different species of minnows, small perch, or the final host itself."

The elucidation of the life history of this parasite has become of real importance since the production of bass had been undertaken by the U. S. Bureau of Fisheries and also by numerous state hatcheries. In the report of the Division of Scientific Inquiry of the Bureau of Fisheries for 1923 there is a note telling of the ravages of *P. ambloplitis* at the U. S. Fisheries Station, Neosho, Mo. Cooper records the presence of the larvae of *P. ambloplitis* in the ovaries and testes, as well as in the other viscera. Recently Bangham (1925) reported on the cestode parasites of bass in the Ohio state hatcheries. He records various larval stages of *P. ambloplitis* and, like Cooper, determines their classifi-

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cation entirely on the basis of morphology. Later Bangham notes, in a mimeographed report on "Parasites as a limiting factor in Ohio fish hatcheries," that but few of the small-mouth breeders spawned. Examination of these showed the presence of the larvae of *P. ambloplitis* in the testes and ovaries. Such inhibitions had been previously noted by Moore (1926). During the summer of 1927, the author carried out an experimental study on the life history of *P. ambloplitis;* a summary of the results of the work is given here. The complete account will appear later. Thanks are due the U. S. Bureau of Fisheries for making this work possible; also to the U. S. Biological Station at Fairport, Iowa, and to the U. S. Fisheries Station, Neosho, Mo.

EXPERIMENTAL WORK

The author assumed a working hypothesis which coincides in the main with that proposed by Cooper (1915): (1) The eggs are eaten by small Entomostraca. In this first intermediate host the parasite migrates from the digestive canal into the body cavity where growth ensues. (2) A small fish eats the Crustacean and the procercoid larva bores its way into the body cavity of the second intermediate host. Here it encysts. (3) The life cycle is completed when this small fish is in turn devoured, thus bringing the larva to the intestine of the bass where proglottid formation takes place.

The writer planned to secure adult black bass from the streams in the vicinity of Neosho, but these bass proved to be infected though not with *P. ambloplitis*. Therefore, attention was directed to the fish in the hatchery ponds. A preliminary examination showed the presence of larval *P. ambloplitis* in some fingerling and yearling large-mouth black bass, pointing to the presence of the adult parasites in some of the mature bass in the ponds. Fourteen adult large-mouth black bass were caught and examined; all were heavily parasitized. Fifty per cent harbored adult Proteocephalidae in the upper intestine, and two included infection with *P. ambloplitis*. In some cases encysted *P. ambloplitis* were also found in the viscera.

The most outstanding feature was the presence of degenerating eggs in the ovary of the bass and this organ was found to be permeated with large, plerocercoid larvae of *P. ambloplitis* ranging from 1.5 to 7 cm. in length. Bass are normally through spawning by the first week of July at Neosho and these larvae were evidently largely responsible for the inability of the bass to spawn. These observations confirm and extend the records of Moore and Bangham already noted.

The eggs of *P. ambloplitis* were obtained in quantity and placed in watch glasses and two large aquaria. Entomostraca from the nearby streams were added to Aquarium I and those taken from the station ponds were added to Aquarium II. By adding the Entomostraca in

quantity it was hoped that a greater variety of possible intermediate hosts would be secured. This mass infection was found to be the most satisfactory method. Eggs were observed and studied in watch glasses and in some instances Cyclops were added, since during preceding experiments it was found that Cyclops functioned as one of the primary hosts. Fifty Cyclops belonging to five species, *C. albidus, serrulatus, prasinus, viridis,* and *fimbriatus,* were examined as controls and no infection was found. Each day thereafter a number of examinations were made. This was continued for 16 days. Only two species of Cyclops, 3 *C. albidus* out of 25 and 3 *C. prasinus* out of 16, ingested the eggs of *P. ambloplitis* and in those cases it was apparently accidental. Several *C. albidus* were placed in a small crystallizing dish containing a spray of water plant and eggs and were studied under a dissecting microscope. After some hours the Cyclops began to browse about the green water plants, readily ingesting protozoa, bacteria, etc. When upon the bottom they encountered the eggs. The outer membrane must serve as a delicacy for this species of Cyclops, for they readily devoured it. It is frequently trimmed off and eaten while the inner membrane containing the oncosphere is rejected. Occasionally even the inner membrane and its contained oncosphere, are eaten, perhaps by accident, and the parasite in this manner reaches the digestive tract of the Cyclops. In the case of both *C. albidus* and *C. prasinus* the oncosphere could be seen in the body cavity four to five hours after ingestion (Fig. 2). It is interesting to note that the infected Cyclops which were examined showed a maximum of only four procercoid larvae. On the other hand Essex working on the life history of Corallobothrium, found as many as 18 to 20 procercoid larvae in a single specimen. No cases of extremely heavy infection were noted; but even in the case of a Cyclops containing four procercoid larvae the movement was somewhat slower than that of an uninfected individual. This lethargy would make the animal an easier prey for the young bass which feed upon Cyclops and other Entomostraca.

Previous to this, systematic examinations had been carried on to determine if possible the age at which the young bass were infected by *P. ambloplitis.* The work done on this phase pointed to the young bass fry as possible second intermediate hosts. This is most likely in localities where the bass were artificially propagated, as Bangham (1925) reported finding the procercoid larvae in bass soon after leaving the nest. At this time the fry in Ohio were feeding primarily upon copepods.

The present writer examined twenty-five bass fry as controls two to four days after leaving the nest and found the food consisting of Cladocera (mostly Bosmina sp.) small Phyllopoda and Copepoda. These occurred roughly in the ratio of 3 : 2 : 1, which is closely correlated

with the ratio of occurrence in the pond where the bass were taken. This means that the fry depend to a large degree upon the available food supply and suggests that the bass will eat any of the smaller Entomostraca available.

About fifty large-mouth black bass fry ranging in size between 1 and 1.5 cm. in length were placed in each of the two aquaria, Number I containing Entomostraca secured from various streams and Number II containing samples of the fauna from the various ponds at the station. Since no results were obtained from Aquarium I it will not be considered further except when used as a control. The fry were added on the third day of the experiment and thereafter were examined periodically. They did not begin to eat the Entomostraca until 5 to 6 days after the stocking of the aquaria. This was determined by examination of the stomach contents of the bass from Aquarium I. When the aquaria were removed to quieter surroundings the fry in Aquarium I began to feed. At this point examinations of the fry from Aquarium II were commenced. Twenty-four hours after the bass began to feed upon the Crustacea, five were examined from Aquarium II and two were found infected. Three days later five more were examined and three fish were parasitized. Procercoid larvae of *P. ambloplitis* were recovered from the liver, mesentery and gonads. In other words, the fry were 50 per cent parasitized with *P. ambloplitis*. The question arises as to whether or not this is a significant percentage. Twenty-five fry of *M. salmoides* taken at the same time were examined as controls and the entire percentage of infection was 20 per cent, only 8 per cent of which was due to *P. ambloplitis*. Furthermore, the controls showed infection with Acanthocephala of 4 per cent and of Ancyrocephalus sp. of 8 per cent. It is significant that the percentage changed but little under experimental conditions with the exception of parasitization by *P. ambloplitis*. Then the general infection percentage was raised to 50 per cent and the infection with *P. ambloplitis* was likewise 50 per cent. In other words every specimen which was parasitized harbored this helminth. Ancyrocephalus sp. was found in 10 per cent of the cases on the gills, and nematodes and Acanthocephala were also found in 10 per cent. Thus it is evident that the only significant increase in parasitization lies in the infection by *P. ambloplitis*.

Ten days after the introduction of the large-mouth black bass fry to the two aquaria the remaining 38 from Aquarium II were transferred to a larger aquarium containing 8 yearling bass of the same species. These had been starved for over 5 weeks and were possessed of a voracious appetite as was shown by the disappearance of all the fry within a period of 12 hours. Three days later four were examined. The first fish had the remains of 4 small fry and was found to harbor five young plerocercoid larvae of *P. ambloplitis*. These were all located

in the intestine just below the pyloric ceca. The next fish had three plerocercoid larvae in the stomach; one was free and the other two attached to the mucosa. The fourth fish was uninfected but the third yearling was infected with 6 plerocercoid larvae, all from the upper intestine. Five were small and measured between 0.2 and 0.3 mm., but one was considerably larger and measured about 2 mm. This possessed the characteristic suckers about 0.25 mm. wide carried upon a scolex nearly 0.6 mm. in width. The vestigial fifth sucker was prominent and measured about 0.26 to 0.27 mm. in optical section. No excretory system was visible but the comparatively large size indicates that this specimen had been in the fish for a considerable period. This is undoubtedly true when one realizes that the parasite next in size measures just under 0.4 mm. in length.

The remaining four yearling bass were left at the U. S. Fisheries Station Neosho until the weather became cool. They were then shipped alive to the Rensselaer Polytechnic Institute where they were kept in cold filtered water. Two of these were examined in the middle of December and two are still unexamined. Those which were examined were parasitized with 1 and 4 plerocercoid larvae respectively. The living larvae measured from 1.62 to 4.7 mm. in length.

Seventeen *M. salmoides* were examined as controls and showed infection of 52.9 per cent. It is significant, however, that *P. ambloplitis* was the only tapeworm found and this occurred in only 17.6 per cent of the fish. Artificial infection based upon an examination of four of the yearlings within a period of three days after the fry were added yielded 75 per cent infection with *P. ambloplitis*. Two more were examined in the middle of December and these yielded specimens of *P. ambloplitis*. This raises the infection percentage to 83.3 per cent for the experimental fish, which is a remarkably significant figure compared with the 17.6 per cent infection in the control bass.

MORPHOLOGY OF PARASITE

The eggs of *P. ambloplitis* were readily secured by placing the parasite in physiological salt solution, or water. It was necessary to study this material alive, for any preserving agent distorts the eggs badly. The six hooked oncosphere or larva is surrounded closely by an investing membrane which in turn is covered with a rather thick and granular secondary one. The outermost membrane is hyaline and takes a variety of forms being commonly round, ellipsoidal or dumb-bell shaped (Fig. 1 a-c).

The eggs of several species of Proteocephalidae were studied and the writer was able to corroborate Cooper's (1915) findings on the eggs of *P. ambloplitis*. He describes another type resembling an equilateral triangle, made up by placing three circles of nearly equal

diameter together. The embryo lies in the upper one. The dumb-bell shaped outer membrane is characteristic of this species. This outer hyaline envelope measures between 26 and 31μ in those that were but slightly oval. Figure 1 b shows the maximum oval shape encountered; in such cases the length of the outer membrane ranges between 58 and 59μ, as is likewise the case of the dumb-bell shaped outer membrane. La Rue (1914) found the outer membrane to be ellipsoidal, measuring 36 to 43μ in diameter. These measurements were probably made upon preserved material, while those of Cooper and the present writer were made on living material. Cooper found the length of this outer sheath varying between 55 and 75μ while in the present instance the measurements of the living material were between 26 and 60μ.

Beneath the outer membrane lies the thick, granular secondary membrane. Within this membrane and between it and the innermost membrane lies a mass containing many small globules. These are sometimes yellowish and variable in size. Cooper suggests their being of a fatty nature.

The innermost membrane, lying closely around the oncosphere itself, is difficult to distinguish; it measures 17 to 20μ in maximum diameter. It may be seen when the egg is broken by the pressure on the cover slip. It has been suggested by some workers that this is the membrane of the oncosphere and not the innermost protective membrane of the egg. However, this view is untenable for embryos liberated by cover-slip pressure do not disintegrate readily and if it were a membrane of the larva it would go to pieces rapidly. Further, the movement of the larvae within these three membranes is another indication of their extra-embryonic nature. The hooks of the embryo were seen to move periodically, as tho tearing at the restraining sheath. According to Cooper these hooks were embedded on a "cone of homogeneous material, the apex of which surrounded the proximal end, slightly swollen in this species, while the base at the surface of the oncosphere was about three times the diameter of the distal end of the main shaft of the hook."

The morphology of the larvae will be described only in so far as it is necessary to complete the gaps in our present knowledge. Cooper (1915) has adequately described the stages from these 0.7 mm. on up and this work will not be duplicated. The larvae recovered 4 to 5 hours after infecting the Cyclops were oval in shape and measured between 26 and 30μ. This is larger than the oncosphere itself where the maximum diameter was only 20μ. Otherwise the larva resembled very closely that within the membranes of the egg. The surrounding membrane encloses the 6-hooked larva. The protoplasm appears vacuolated (Fig. 2) and the six hooks are still functional. These move from time to time as the procercoid elongates in an amoeboid fashion. This move-

ment appears typical of the larva at this stage of development, and the movement of the hooks suggests their use in penetrating the digestive tract of the Cyclops.

By the fifth day the larva has attained a slightly larger size, measuring between 40 and 52μ. At this stage the parasites showed a number of excretory granules and the protoplasm appeared denser. The embryonic hooks of the oncosphere were not in position, but were scattered throughout the protoplasm. One specimen recovered from *C. albidus* showed one pair and two other hooks scattered through the protoplasm and a smaller larva, measuring about 34μ in length, recovered from the body cavity of *C. prasinus* seven days after the eggs were added to the culture showed one pair of hooks and a single odd one. It is probable that the hooks function only in the penetration of the gut wall of the Cyclops and thereafter are cast off.

Parasites were recovered from the Cyclops up to the sixteenth day of infection. The changes occurring in the larvae examined since the fifth day are as follows: (1) The larvae have taken a definite shape and can be readily recognized as procercoids. The region of scolex formation is over twice as broad as the remainder of the parasite and measures about 40μ as compared with 17 to 20μ for the posterior regions. (2) The excretory granules which lie scattered throughout the procercoid have increased in size and quantity. They are not so numerous in the region where the scolex will form, and are concentrated about 5 to 10μ from the outside in two rows. (3) There is a decrease in the body length compared with the more rectangular, less highly developed larvae which were recovered on the twelfth and thirteenth day of inoculation when those taken measured about 0.12 to 0.13 mm. in length compared with a scant 0.1 mm. for larvae examined upon the sixteenth day. (4) The anterior portion of the body has invaginated and within it can be seen the primordia of the suckers (Fig 3). This invagination occurs about the twelfth to thirteenth day and takes place before the suckers form. The procercoid larvae develop very slowly, for by the sixteenth day of infection they measure only 0.12 mm. and developing suckers appear along the edge of the invagination. At the distal end of this may be seen the developing end organ, and throughout the body, posterior to the scolex, are the excretory granules characteristic of larval forms.

On August 5, 1927, eight days after the fish were added, and the eleventh day of the experiment, procercoid larvae were recovered. Five fish were examined from Aquarium II and two of these were infected. In the first infected specimen examined one larva was attached to the stomach wall and was evidently in the process of penetrating it. The larva measured about 52μ in length by 25 to 32μ in width. The body appeared dense and a number of excretory granules were apparent.

Two of the embryonic hooks were still in the protoplasm and were apparently without means of attachment for they moved aimlessly about as the procercoid expanded and contracted. At one end an invaginated fold marked the region of the scolex formation. Likewise the protoplasm in this region was more dense and was marked by the scarcity of the excretory granules. The end organ was evidently just beginning to form at the distal end of the invagination chamber for this region appeared more dense and the outline of this organ was in evidence. Other larvae recovered measured up to 67μ in length by 15 to 20μ in breadth when expanded; these were recovered from the liver (Fig. 4). The other four embryonic hooks may have been present but were not visible at the time the parasite was studied.

Three days later five more of the large-mouth black bass fry from Aquarium II were examined and three were infected. The first one examined yielded a single specimen from a mesenteric cyst. The cyst was ovoid and measured 0.25 mm. in length and 0.11 mm. in maximum width. The cavity of the invaginated scolex could be seen as well as the granular, highly refractive excretory deposits (Fig. 5). Upon opening the cysts a young procercoid larva was recovered. This was by far the largest specimen encountered, measuring nearly 0.2 mm. in length. Likewise it showed more advanced scolex formation. The fifth vestigial sucker, or end organ, was beginning to form and was characterized by its position at the distal end of the invagination chamber. On either side could be seen the primordia of the smaller, functional acetabular suckers. The next infected fish yielded three procercoid larvae all taken from the liver and all at the same stage of development. They ranged in size between 0.29 and 0.32 mm. long and 0.1 to 0.12 mm. wide. The entire protoplasmic mass was slightly denser than the one taken from the mesenteric cyst. The fifth sucker and the functional ones appeared along the invagination chamber. Likewise there were a number of excretory granules and at this stage no indication of a system of excretory tubules was evident. Observations point to the formation of these only after a considerable period of encystment. The third infected specimen gave the most interesting results of the lot, for in the gonads was found the most highly developed parasite. It was slightly longer and broader, measuring 0.3 mm. by 0.15 mm., respectively. The end organ and the suckers showed up clearly and measured 54μ and 25 to 30μ in diameter, respectively (Fig. 6). This differs from the figures recorded by Cooper (1915) where he cites 58μ as the diameter of the end organ and 84μ the suckers. A study of sectioned material confirmed this for the diameter of the suckers increased from 25 to 30μ in a 0.2 mm. larva to 124μ in larvae 1.3 mm. long, while in a 1.8 mm. specimen the maximum diameter of the suckers was 131μ. The end organ, on the other hand, shows an increase

in size from 50μ in a 0.2 mm. larva to 155μ in one that is 2.9 mm. in length. This does not agree with Cooper's figures of about 230μ in one the same length. Later this structure degenerates and decreases in size, but not until the parasite is more mature. Sections also show two thin rows of longitudinal muscle fibers extending from the region of the scolex posteriad. In a larva measuring 0.6 mm. in length a caudal vesicle measuring 50μ was encountered. This vesicle was forked for a distance of about 15μ, the forked portion being lined with a continuation of the cuticula. Cells are found grouped about the vesicle in a manner noted previously by Cooper, and in the region of the scolex are other vessels which anastamose with the more posteriad ones, though they are more closely compressed due to the invaginated scolex. In other respects this helminth did not differ materially from those taken from the liver and mesenteric cysts.

Presumably these parasites were all taken into the body of their respective hosts at about the same time. Yet the one taken from the gonads was more advanced. This may mean one of several things: (1) The parasite may have been eaten by the Cyclops soon after the experiment was started, its greater size being due to the additional time passed in the first intermediate host before being eaten by the fry. Such a condition would have given it more time in which to develop and grow. (2) Another possibility lies in the early ingestion of the Cyclops host by the bass fry. (3) The third suggestion is based upon the location of the parasite within the second intermediate host. Embryological studies show that all eggs are rich in nutritive material. It would seem quite probable that this location would be very favorable to the parasite, for it would be surrounded by a great mass of eggs, containing considerable quantities of deutoplasm.

Since the morphology of larvae measuring over 0.7 mm. has been adequately described by Cooper (1915), the present author has not dealt with this aspect of the problem. However, certain differences are apparent between the several stages and these will be briefly summarized. The parasites from the yearlings all possessed evaginated scolices. The suckers ranged in maximum diameter from 25 to 30μ in a specimen about 0.2 mm. in length. The end organ or the fifth sucker likewise was apparent and measured between 50 to 75μ. All the larvae recovered the first three days of yearling infection with the one exception already noted, ranged in length between 0.25 mm. and 0.4 mm. and in width from 0.14 to 0.155 mm. (Fig. 7). There was no external indication at this stage of a tubular excretory system, but this was visible in sectioned material. The excretory products were precipitated in crystalline form and scattered throughout the body posterior to the scolex. These excretory granules are characteristic of the larvae until the excretory system proper has developed. This occurs at about 4 mm.

when they probably become dissolved and are carried off by the excretory tubules. The parasites recovered 4 months after infection were between four and eleven times longer than those taken the third day of yearling infestation. One specimen 4.18 mm. in length (Fig. 8) was sectioned and gave the following data: The thickness of sucker (base to back) 0.405 — 0.054 mm.; the diameter of sucker 0.148 — 0.155 mm.; the depth of sucker 0.047 — 0.074 mm. The excretory system was forming as two pairs of small canals and their ramifications were found extending from the excretory vesicle to the scolex.

The plerocercoid larvae recovered in December differed from those recovered the third day of infection in the following respects: (1) Externally they were longer and broader than any previously examined, and the size of the different parasites varied, due to the differences in the environment in the respective fish, which would vary according to the amount and type of food taken in. This food consisted largely of small minnows (*Notropis hudsonius*) 25 of which had been examined to determine the parasites and percentage of parasitization. Twenty-four per cent were infected and these only with nematodes, so it was safe to feed them to the yearling fish. Naturally in using live minnows for food some of the bass would eat more than the others and this would therefore affect the environment in which the parasites (*P. ambloplitis*) existed. At any rate the parasites varied between 1.62 mm. and 4.7 mm. in length, and in width from 0.128 to 0.37 mm. (2) The scolex was more prominent than in the smaller forms recovered after three days infection. And likewise the diameter of the scolex was greater, measuring between 0.405 and 0.438. (3) The excretory system evidenced some development, for the parasites recovered in December showed a more advanced system of longitudinal excretory tubules, than those recovered the third day. (4) The fifth or vestigial sucker showed considerable growth; in those examined within the first three days of yearling infection the diameter of this structure ranged between 60 and 75μ compared with 0.216 mm. in the form taken this December. All the organ systems show evidence of further development and specialization (*cf.* Cooper, 1915).

DISCUSSION

Recent workers have been forced to face the possibility that the infections secured under artificial experimental conditions may not be typical of the organism in nature. In a life history study where morphology is employed to supplement the experimental phase and the primary emphasis is placed upon the experimental method, the conditions must be controlled accurately. This has been attempted by the following means: (1) Subjecting the control and experimental organisms to identical conditions as far as possible. Thus, any variation which

would occur in one would likewise be found in the other. For example, the bass fry used in the experiment were taken from only one school of bass. They were retained in a single aquarium until the experiment was started and then 25 were examined as controls. (2) The use of a sufficient number of specimens as controls. The writer has tried to use 25 or more individuals in each case. With the Crustacea 50 control Cyclops were examined. Twenty-five large mouth black bass fry were used as controls. (3) Great care was exercised in keeping the yearling large-mouth black bass shipped from Fairport free from infection after their arrival at Neosho. They were placed in a cement tank which was fed directly from the spring. A careful and periodic examination was carried on to insure the absence of Crustacea. Some, however, were present in the tank. The last of June these fish were transferred to the troughs in the tank house and kept there without food for over five weeks in the cold spring water which had a constant temperature of 59° F. (4) The identifications made in the field were subsequently checked in the laboratory by means of sectioned material and totos. The evidence obtained indicates that two species of Cyclops, *C. albidus* and *C. prasinus,* function as the first intermediate host. The experimental infection percentages were 12.3 per cent and 18.7 per cent, respectively. During the course of the summer records were kept on the examinations of 101 Cyclops including five species. Only two of these were infected and only one each of the species *C. albidus* and *C. prasinus*. This was only 4.7 per cent of the 21 *C. prasinus* examined and 3.1 per cent of the 33 *C. albidus,* the other three species were uninfected.

It is possible that some other invertebrate may normally function as an alternative first intermediate host. For example, Bangham (1925) reports the discovery of a young plerocercoid larva encysted in *Hyalella knickerbockeri.* This he suggested might prove to be an intermediate host of this parasite. Since that date he has apparently confirmed it for in an account of the life history of *P. ambloplitis* read before the 1927 meeting of the American Fisheries Society, he reports this as one of the intermediate hosts along with *C. leuckarti.* It would seem, therefore, that *P. ambloplitis* has four known first intermediate hosts, *C. prasinus, C. albidus, C. leuckarti* and *H. knickerbockeri.* The last named form cannot be eaten by fry as small as those which feed upon Cyclops This would postpone infection until the bass had reached a length of 8 to 9 cm., in other words nearly yearlings.

One question concerning the determination of *P. ambloplitis* should be considered at this point. This would be a comparatively simple matter if this particular parasite were the only Proteocephalid recorded from the black bass. Unfortunately this is not the case, for although in 1914 La Rue reported *P. ambloplitis* as the only Proteocephalid from the family Centrarchidae, other workers have recently added to this list.

Bangham (1925) after studying the parasites of the black bass in Ohio, records *P. pearsei* from both the large and small-mouth black bass, and also *P. fluviatilis* and *P. osburni,* both new species from Ohio, from *M. dolomieu.* According to Bangham, Needham and Sibley found *P. exiguus* in *M. dolomieu* from Lake George, N. Y. Bangham likewise reports the presence of two larval Pseudophyllideans, both from the small-mouth black bass, *Triaenophorus nodulosus* encysted in the liver and *Bothriocephalus claviceps.* This clearly complicates the problem of accurately distinguishing *P. ambloplitis* from the Proteocephalids mentioned above.

According to La Rue (1914) *P. ambloplitis* had only been reported from fish living in waters draining into the Red River of the North or the St. Lawrence. Since that date Bangham (1925) has reported taking two infected bass from Lake Chautauqua, N. Y., as well as others which harbored larval cysts in the viscera. This evidence points to the establishment of this parasite in the Ohio drainage, for even though not yet reported it may eventually become established there unless the planted bass are free from this cestode. Likewise the distribution of infected fry and fingerlings will eventually lead to the establishment of this parasite in the Missouri and Mississippi River systems. There is little doubt that hatcheries are distributing bass infected with this helminth. Furthermore, since *P. ambloplitis* has been reported from four hosts, *Ambloplites rupestris, M. salmoides, M. dolomieu* and *Amiatus calvus,* it stands an excellent chance of surviving wherever and whenever introduced.

The eggs used in these experiments were readily distinguished by their characteristic dumb-bell shape which was first described by Cooper (1915). An examination of the fish at the hatchery and those taken from the streams revealed the presence of several other species of Proteocephalids. These were distinguishable by various morphological characters as well as by the shape of the eggs. But the eggs furnish adequate criteria for distinguishing in the field *P. ambloplitis* from other Proteocephalids.

On the other hand the larvae could not be distinguished until such time as the suckers had started to develop. This was accomplished by the third day of infection in the Cyclops, at which time the larvae of *P. ambloplitis* show the growth of that peculiar and characteristic organ called a vestigial fifth sucker by La Rue (1914). It appears early in the development of this parasite and is not apparent in similar stages of other species encountered at Neosho. This larva bears some superficial resemblance to the larva described by La Rue (1909) for *P. filaroides* and probably *P. lönnbergii,* but they were not encountered at Neosho. During this work no tapeworm larvae, except those of *P. ambloplitis,* were found in the body cavity of the bass and Cyclops.

The two characteristics noted serve to distinguish *P. ambloplitis* from other members of the genus. Even after the scolex is evaginated, the vestigial fifth sucker is in evidence where it may be seen embedded in the scolex near its distal extremity. Observations made upon young adults show the presence of this structure in living material until well after proglottid formation has taken place. Even when this structure begins to decrease in size and undergo degeneration, it differs from the others encountered in the black bass because it is nonfunctional, deeper set and larger than the fifth sucker found in the five suckered forms. The only time then at which the identification of the larvae would be in doubt is during the first three days of development, i. e., from the time of ingestion by the Cyclops until the invaginated scolex begins to form in the procercoid larva and the vestigial fifth sucker appears.

One of the paramount questions which must be decided is whether or not this cycle as worked out is a typical and normal one. The problem was attacked from the practical angle in order to determine what methods should be pursued to secure its elimination. The writer feels that this life history as worked out experimentally may not be the only picture, nor necessarily the typical picture of the cycle in nature. The time was short and the larvae were not as fully developed in the Cyclops as might be expected normally; a like situation might have prevailed in the case of the yearling infection. As a fish grows to maturity it may harbor this larval form for years. Thus, adult breeders have been found to harbor in the reproductive organs pleurocercoid larvae of *P. ambloplitis* 7 cm. long. These fish were in the third summer or older. Should this fish be eaten by another, would these larvae develop or have they passed the point where proglottids may be formed? And in the experiments since the fry were eaten so soon after acquiring the parasite, did it develop as much as it might have? Presumably, there must be a wide range during which the parasite may be acquired, but conclusive proof is wanting on this point.

Another interesting problem is the rate of growth from the time the plerocercoid larvae are introduced into the digestive tract until proglottid formation takes place. This naturally would depend to a large degree upon the amount and quantity of the food. The experimental yearlings which were infected had been starved for a considerable period and were not fed often since their arrival at Troy.

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Explanation of Plate XI

Fig. 1 a, b, c.—Eggs of *Proteocephalus ambloplitis.*

Fig. 2.—Larva recovered from body cavity of *C. albidus* 4 to 5 hours after ingestion.

Fig. 3.—Procercoid larva recovered from body cavity of *C. prasinus;* 16th day of experiment.

Fig. 4.—Procercoid larva recovered from stomach of *M. salmoides* fry; 2nd day after fry began to feed, 11th day of experiment.

Fig. 5.—Encysted procercoid larva in mesenteric cyst of *M. salmoides* fry; 5th day after fry began to feed, 14th day of experiment.

Fig. 6.—Larva recovered from gonads of *M. salmoides* fry; 5th day after fry began to feed, 14th day of experiment.

Fig. 7.—Pleurocercoid larva, showing evaginated scolex, from intestine of yearling *M. salmoides;* 3 days after fry were fed to yearlings, 16th day of experiment.

Fig. 8.—Cross section through scolex of pleurocercoid recovered 4 months after infection of yearlings.

The lines in figures 2 and 4 have a value of 0.02 mm.; in all the others, 0.05 mm.

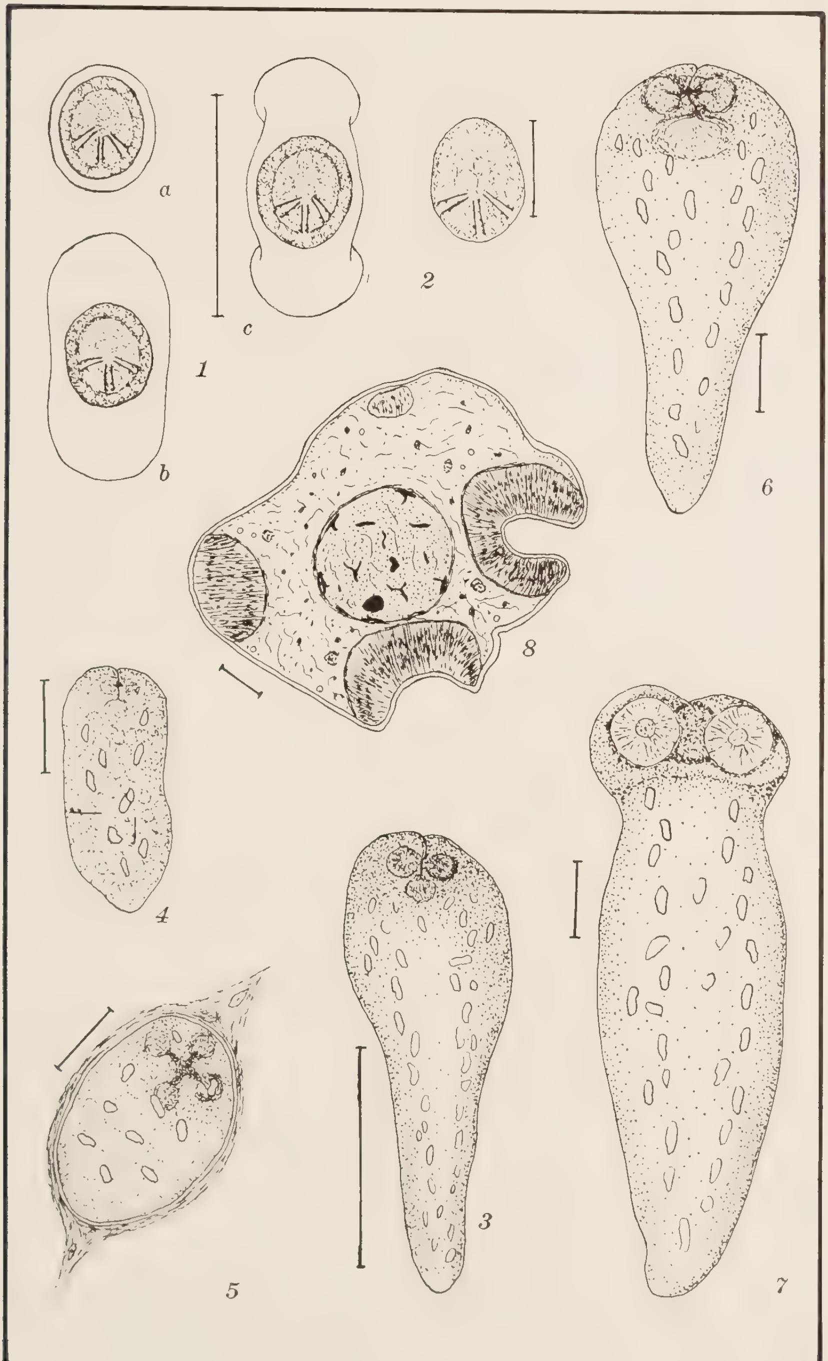

PLATE XI

METHODS OF COLLECTING AND REARING THE IMMATURE STAGES OF TABANIDAE (DIPTERA) *

CORNELIUS B. PHILIP

BOZEMAN, MONT.

Horseflies and deerflies have constituted one of the serious insect scourges in Minnesota since the days of the pioneer farmer and lumberman. They are especially troublesome in early summer in the northern parts of the state where muskegs and the broken, timbered nature of the country offer suitable places for breeding and protection for the flies.

With the idea in mind of obtaining more definite information leading toward alleviation of this scourge, preliminary studies were undertaken to ascertain the species involved and, as far as possible, the manner of their development. Some difficulty was encountered in developing a technique for making suitable biological studies and, in view of the recent valuable contributions by Isaac (1924), Stammer (1924) and Cameron (1926), it seemed worth while to record the methods which were found suited to the needs in Minnesota.

Rearing technique for the immature stages has been variously developed in a number of widely scattered countries. The first account of the early stages of any tabanid was published in Europe by Degeer in 1760. Since the larvae of the species (*Tabanus bovinus* L.) discovered by him were terrestrial and also were nearly mature, he required only a jar of damp earth for completion of their development, although he lost a number through cannibalism. This procedure was later modified by Hine (1906) in the United States and improved by Mitzmain (1913) in the Philippines for rearing semi-aquatic larvae. Both advocated separting the individual larvae because of their cannibalistic tendencies. Their "jelly-glass method" was employed by Jones and Bradley (1923) in rearing Louisiana species. The investigations of Webb and Wells (1924), also in the United States, and of Cameron (1926), in Canada, are typical of some excellent work using modifications of this method.

Hart (1895) does not mention the rearing of the tabanid larvae collectively in his Illinois investigations, but his descriptions of using wide-mouth and battery jars in which the natural environment was reproduced as nearly as possible, would indicate that he did so rear them. He makes no mention of their cannibalistic habits. King (1908)

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* This paper is based upon investigations carried on at the Division of Entomology of the Minnesota Agricultural Experiment Station, and is a part of a thesis to be submitted to the faculty of the Graduate School of the University of Minnesota. The problem was proposed by Dr. W. A. Riley, whose suggestions have been a continuous help, and to whom my sincerest thanks are due.

and Neave (1915) working in Africa, and Patton and Cragg (1913) in India favored rearing the larvae also more or less collectively in basins or trays of various sizes containing mud or sand with growing vegetation and water to simulate natural conditions.

Marchand (1907), again in the United States, using test tubes about seven and three-quarters inches long containing a roll of wet filter paper, developed the first technique for making satisfactory observations on the growing larva but failed to carry any individuals through from egg to adult by this method. He found these containers satisfactory for rearing larvae from 4 to 40 mm. in length, however, and pupae from fourteen species of Tabanidae were obtained by him using this procedure.

Isaac (1924), rearing horseflies in India, found Marchand's method unsatisfactory especially for newly hatched larvae. He devised another method for making accurate observations on the larval instars which is essentially an original modification of the "jelly glass" technique. Using the methods he proposed, he has subsequently recorded complete life histories for several species, including the first accurate observations on the number of larval instars. The improvement consists in the use of smaller amounts of clean sand with moisture according to the needs and in the use of an excess of water while making the observations. The containers employed are small beakers for the younger and, for the older, glass jars with gauze and petri dish covers, in all of which the sand is kept tilted to allow for a moisture gradient from top to bottom of the sand. These vessels are kept in glass tanks containing half an inch of water over the bottom and a wire gauze cover if desired. Food, generally freshly killed fly maggots, is easily introduced and observations on the condition of the larvae are made by adding a surplus of water and shaking slowly, when the larva, shed skins or any food and refuse roll out on top of the sand. The sand is again allowed to become tilted on one side of the container, the excess water is poured off and the larva allowed to crawl back into the sand. Stammer (1924) in Europe, reared his larvae in glass dishes containing sand and a little moss. These were fitted with heavy covers to prevent the larvae from pushing the lids off and escaping.

Methods have been variously modified and gradually improved, and the lack of uniform methods in the various countries may not indicate merely the lack of a stabilized and satisfactory procedure but in many cases it would seem to indicate rather the development of a technique suited to the needs of the investigator and adapted to the environmental conditions in which he works. Obviously the great handicap in rearing cannibalistic species is the inconvenience involved in caring for the individuals separately. This, of course, is offset by the greater facility of making and keeping an accurate record of observations in the

separate handling of the early stages. However, this facility is not attained in the methods employing sand or soil as the substrate, in which the awkwardness in separate handling is not compensated by ease of observation with little disturbance of the subject. The collective procedure developed by Hart or King and expanded by Patton and Cragg, is also cumbersome and of little value for individual larval records.

The technique developed by Isaac has "facilities for the close observation of the larva" in its favor over the older methods, but it still leaves much to be desired in the way of facility for detailed study during such processes as molting or pupation. Furthermore, it retains awkward features in the handling and rearing of more than a few of any series of individuals at any one time. Especially is this apparent in countries having greater seasonal extremes allowing for only one brood per year and thus necessitating holding the larvae in the laboratory through the winter. In India where two and three broods of horseflies are the rule, the duration of the larval and pupal stages may be less than a third that of those species found in climates with more rigorous seasonal extremes.

The test tube method developed by Marchand has the advantages of allowing fairly undisturbing, detailed observation, especially of such activities as eating, molting or pupation—as some larvae in the series are nearly always between the paper and the glass—without the disadvantages of bulkiness when a considerable series of larvae is being handled. It has the objections raised by Isaac of being an environment very unlike that of natural conditions and of being impracticable for newly hatched larvae. Cameron has also found it unsatisfactory for both young and mature larvae, because of frequent death by drowning or infection from moulded pieces of meat supplied as food. He finds that larvae of Chrysops fail to thrive and invariably die unless transferred from the test tubes to a more suitable environment.

PROCEDURE ADOPTED UNDER MINNESOTA CONDITIONS

Most of the above methods were tried at Minnesota and a technique was developed embodying certain ideas from a number of them which seemed most satisfactorily adapted to the local needs. As the rearing was necessarily carried on incidental to class work and other activities, that procedure which facilitated observation with the least demand on time and space, was found to be a combination in the main of Marchand's method with modifications of other ideas.

Instead of test tubes and rolled filter paper, which shreds and wads up badly after wetting, homeopathic vials of about two and one half inches by five eighths or six eighths inches in which short strips of

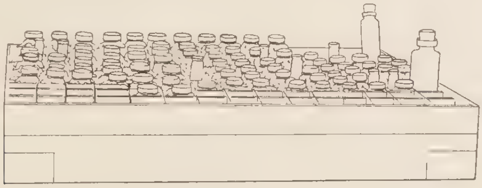
ordinary, rather tough, paper towelling were rolled, were found to be most convenient. Squares of cheesecloth held in place with rubber bands provided effective covering (Fig. a). Even the large larvae of *Tabanus stygius* Say, 45 or more mm. in length, seemed to do quite as well in these small quarters as did larvae of the same lot kept in jelly glasses with sand. The later larval instars of several species were compared in this manner and while not known to be of the same brood, those in the smaller quarters seemed equally content, and to develop equally fast on the average with their fellows in the jelly glasses so long as they had plenty of food and moisture. When the larvae are first placed in the vials, when they are ready to pupate, or when the conditions in the vials are unfavorable, such as lack of moisture or food, they are observed to become a bit restless, and two or even three thicknesses of cheesecloth squares are not effective in keeping an occasional larva from spreading the mesh and escaping. In this case, however, the tell-tale hole is quickly noted and the escaped larva returned as the later instars, at least, withstand drying for a day without apparent ill effects in most cases.

The small area opening through the meshes of the cheesecloth allows the free access of air but cuts evaporation to a minimum so that there is practically a saturated humidity in the vials, as shown in figure b by a cut in the paper roll in one of the vials; just enough water is necessary in the bottom to keep the paper wet. If the inner end of the paper strip is looped in reverse direction as it is rolled for placing in a vial, a crosswise partition results which aids small larvae materially in climbing up the sides and little trouble from drowning has been encountered. The rounded bottoms of the test tubes, and the tight clinging of the wet roll to the sides probably contributed to the invariable death reported by Cameron for Chrysops larvae, as the larvae collected in the field of ten of the eighteen species occurring in Minnesota have been reared in homeopathic vials.

The original carton occupied by the vials makes a very convenient container for the series of vials with larvae when the edges are trimmed down so that the tops of the vials protrude to facilitate handling. The partitions separating the original rows of vials are slit to allow for crosswise partitions forming a rigid lattice work similar to an egg carrier (Fig. a). Groups of larvae can thus easily be kept in series, and the exuviae from molts or pupation, as well as preserved larvae or pupae, can be kept readily available in the series if desired. Shed skins should not be allowed to remain in the moist vials long as they deteriorate rapidly.

It was found that fewer larvae escaped after becoming adjusted to their surroundings when the vials were kept dark by the partitions of

the box, than when left out where light easily penetrates the glass and paper towelling. Although they seem to avoid as much light as possible, their chief response appears to be thigmotactic. They seem to desire as many points of their body in contact with something as possible whether it be sand or folds in the paper towelling. For this reason tin salve boxes with moist blotting paper discs, often used for cutworm rearing, proved an unsatisfactory method of rearing for tabanid larvae. They would insist on crawling under the blotting paper next the can, making them continuously troublesome for observation, in addition to the annoying rust which developed after a time. Furthermore pupation frequently resulted in an imperfect pupa by this method, and food, especially earthworms, soured badly in the cans.



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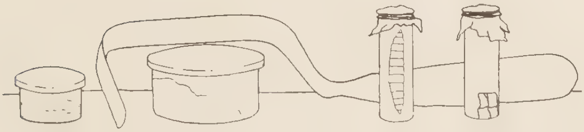

b

Explanation of Text Figures

Text figure a. Convenient method using homeopathic vials for rearing tabanid larvae in considerable series. Vials for preservation of exuviae and dead specimens may be included in their proper series if desired.

b. Collecting and rearing apparatus. Horticultural hand weeder for field work; two sizes of stender dishes for early instars; homeopathic vials for later instars, one containing mature larva of *Tabanus stygius*, the other with paper roll cut to show depth of water for moisture maintenance and reverse fold making central partition to aid smaller larvae in getting off bottom

Earthworms were found to be inferior to fly maggots in point of handling. A sour earthworm is rivaled in stench only by a putrid snail as far as larval horsefly food is concerned, and while such souring of food material seems not to affect the tabanid larva particularly, unless left for several days, it is extremely offensive to the investigator, necessitating repeated changing of the paper. The infections reported by Cameron while using this method, were only infrequently met with during a large series of rearings, and in most cases it was difficult to ascribe death to soured food rather than to injury in collecting or hand-

ling. Freshly killed fly maggots, introduced as food seldom foul the vial and are readily consumed, as the tabanid usually either completely eviscerates a maggot before quitting it, or leaves it practically untouched. In either case it is readily disposed of without changing the paper. In inducing newly hatched larvae to start eating, it is often advantageous to kill the maggot by cutting it in two, as the throbbing parts offer a tempting morsel to the uninitiated young larva. For the older larvae, maggots are best killed by drowning. Live maggots offered for food may retaliate the attack of the tabanid, often resulting in injury to the latter also.

The prepupal stage is indicated by the quiescence of the larva, its continued refusal of food, and the appearance of spiracular projections laterally between the first and second segments. After pupation and sometime before the adult emerges, as indicated by the darkening of the pupa and appearance of eye colors, the paper towelling should be pushed down around the pupa leaving the anterior end projecting with room in the top of the vial for the adult to emerge and hang on the cheesecloth to stretch its wings. Most pupae will insist on following their instincts to try to wiggle upwards anticipating emergence. It was soon found that the use of filter paper, as suggested by Marchand, results in a tangled mass of shreds among the abdominal spines of the pupae due to this writhing instinct, thus obscuring many of the pupal characters for study after emergence. Paper towelling is much superior in this respect.

Newly hatched larvae of Tabanus were found to be most conveniently reared and studied in small stender dishes having tight fitting covers. This latter point is important as otherwise valuable larvae may be lost through rapid evaporation in the small dishes. Another costly experience was gleaned when several young larvae were lost because of their sensitivity to high temperature from too prolonged exposure to a desk light during detailed observations. Two sizes of dishes were employed (Fig. b). The first or second instars were placed in the smaller (40 mm. in diameter by 20 mm. deep) and later in the larger (60 mm. by 30 mm.) until transfer to the vials during the last three or four instars.

A dead piece of water soaked leaf, or ordinary paper towelling was added to the dish together with just enough water to cover the larva.* The leaf supplied a place under which to hide, and seemed to satisfy the apparent need of the larva for some contact before it would become quiet and cease restlessly moving about. While it is busy feeding on bits of

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* Following Isaac's suggestion, it may be found more convenient for maintenance of moisture, to bank a little clean sand on one side of the dish. It is a little more troublesome for keeping observations on a series of young larvae, however, and where both sand and a piece of leaf or paper are used, the larva is as apt to be found under the latter as in the former.

earthworm or fly maggots supplied as food, observations can be made with either the binocular microscope or the low power of the compound microscope, as desired. Changes in Graber's organ were readily studied under the low objective when the larva was found resting quietly with only the anterior segments covered by the leaf as frequently happens. All newly hatched larvae of Tabanus subjected to this treatment were those having swollen tracheal trunks, but those apparently terrestrial species with more slender tracheal trunks should do equally well. A convenient time for transfer to the homeopathic vials was found to be between the third and fourth molts.

Newly hatched larvae of Chrysops were placed under various modifications of this technique, as well as a bulk plan in which the larvae were placed in a large photographic tray, 10 by 12 inches, with sand, muck and debris at one end, and water at the other, but in no case did they appear to continue development beyond the second instar although some lived for nearly a month. *Chrysops fulvaster* O. S. was the only species of deerfly showing any tendency to cannibalism in the early instars, but they also succumbed finally, and as both Stammer and Cameron point out, the normal food of the genus is probably the organic matter to be found in the muck in which they live. Nearly all the last instar deerfly larvae found in Minnesota refused the animal food offered excepting an occasional larva of four species that accepted pieces of oligochaetes. It is still an unanswered question as to why they cannot be reared from the egg under laboratory conditions.

All deerfly larvae collected in the fields, above the pond margins, have been of the last instar. Two collected with an Eckman dredge of one fourth cubic foot capacity, October 16, 1924, in the bottom of a pond around the base of Sagittaria on which egg masses were frequently laid, were the only ones not yet in the last instar. The position of Chrysops egg masses on plants over water several feet from shore and the above occurrence of larvae too far under the surface to be able to reach air, would seem to indicate the possibility of their obtaining oxygen either from that dissolved in the water, which is unlikely, or in a manner somewhat similar to that followed by the aquatic larvae of *Mansonia perturbans* Walk. and the beetle larvae of the genus Donacia, by tapping the air spaces of plants with their stigmatal spines. In this case a different rearing technique would have to be evolved in all probability.

Second instar larvae of Chrysops, under observation in stender dishes were never seen to take air, although the more mature larvae could frequently be observed taking air by protruding the stigmatal spine through the surface film. In spite of rather diligent search in the field very few Tabanus egg masses were found, although those of Chrysops were rather plentiful. The bulk of the biological studies at Minnesota was therefore with larvae collected in the field, especially in the spring,

when the nearly mature larvae are more or less concentrated at the margins of aquatic environments with less vegetation to interfere with the collecting. Following extensive spring collecting as many as three hundred thirty separate larvae have been taken care of daily using this procedure without interfering with routine classwork and other regular activities. This vial method also proved most convenient in continuing larval records of the later instars during an auto trip of several days across three states. By keeping the series of vials in their regular container under the seat, daily observations were made very readily and no breakage occurred.

It has been objected by many investigators that any method of rearing, similar to Marchand's, which does not employ an opaque substratum such as sand or earth does not furnish an environment suitable for normal development of tabanid larvae. Considerably more data should be gathered than are now available before the validity of this objection can be fully tested. A few observations during the studies at Minnesota may be enlightening, however. It was found that newly hatched larvae reared in a dark room for over five months and only subjected to artificial light for a short period once in two or three days to make observations showed no greater differences in rate of development than could be ascribed to the ordinary variations found among individuals of the same egg mass and reared in glass stender dishes under normal light conditions near a window in the laboratory.

As previously pointed out, the jelly glass method for rearing larvae found in the field was discarded because no advantages in development could be detected over those reared in the more convenient vials without earth, muck or sand. Since the young larvae reared in stender dishes are as frequently found between light pieces of paper and the glass sides of the dish as in the sand, and older larvae placed in dark cans still insist on crawling under something, the indication seems to be that a considerable share of their restlessness may be in the line of thigmotactic responses. The matter of whether it be sand or folds of paper would seem of less importance than was formerly thought.

COLLECTING METHODS

Collecting the immature stages of tabanids is sometimes a difficult task, but one which becomes easier with experience. Pupae were never found in any abundance except twice in the case of *Chrysops mitis* O. S. in the late spring. Larvae are most abundantly encountered in Minnesota during the spring months when all the later instars, but especially the mature larvae are concentrated along the margins of various aquatic environments. It was found that a considerable series of larvae of certain species of both Tabanus and Chrysops were rather easily obtainable after becoming acquainted with suitable localities.

Steep artificial embankments along one side of a pond, such as railroad or highway grades where dirt rather than rock or cinders had been used to make the fill at least one season before, were found to be ideal collecting places for certain species. This was probably due to the steep moisture gradient at the margin of the pond causing a concentration of the larvae rather than any preference being shown for that particular side of the pond by the ovipositing female or migrating larvae. In fact, it is likely that the greater numbers of larvae occur along the more gradual margins offering a much increased area with the proper moisture conditions. The difficulties involved in collection of larvae, however, rapidly increase with such increased area where the vegetation, roots and debris all serve to hinder a thorough examination of the soil.

Mature larvae and pupae of *Tabanus lasiophthalmus* Macq. were found frequently in the ant-hill hummocks scattered over boggy land and some have been found in rotten logs a considerable distance from open water. Larvae of *Tabanus stygius* Say have also been found in rotten logs near the margin of certain ponds in addition to their occurrence in the muck along the edges. Only one specimen of an undoubtedly terrestrial tabanid has been found. This occurred amongst the leaf mold of a basswood-maple forest floor.

The sieve method of straining out tabanid larvae advocated by Marchand (1917) and others was quickly discarded as too slow and injurious for practical purposes in Minnesota. A ball of mud immediately formed which was slow or impossible of dispersion and frequently injured larvae against the meshes of the sieve. Cameron (1926) discarded this method for similar reasons. Some have advocated the transportation of the soil to the laboratory where it is treated and the larvae recovered. Of these, the procedure evolved by Stammer (1924) seems to offer the best possibility. After separation of the soil proper from the detritus by washing, the latter is placed in a strainer over a shallow pan of water, as the layer of detritus dries from the top the larvae drop into the pan below. The smaller larvae are recovered from the separated soil by sieving. The time consumed and results obtained do not warrant such a procedure in Minnesota, however, as many samples would frequently have to be so treated before a few larvae were taken, except in the few places mentioned above where certain species are rather numerous.

Cameron obtained best results with a "vertically" (probably right-angled) tinned garden hand fork. At Minnesota an ordinary garden trowel was used for a time after discarding other methods. The handiest instrument both for sampling and for intensive examination of the soil, especially in weedy situations along the margins of sloughs, proved to be a horticultural hand-weeder (Fig. b). It is remarkable how few

larvae are injured with the use of this instrument, and how many larvae of all sizes can be located especially as the eye becomes accustomed to the task. It has since been used for all types of soil insect work, in the study of cutworms, wireworms, etc., and has been found particularly adaptable where an ordinary trowel proves cumbersome. The purpose accomplished by Cameron's garden fork and by the weeder used at Minnesota is the same in both cases, namely, the rapid examination of considerable territory. Many more larvae are obtainable in this way than can ever be found in an equal time by the more thorough sieve method although it is granted that Marchand's or Stammer's methods will have to be used where quantitative sampling is desired.

A procedure found particularly adaptable to the collection of Chrysops larvae was that of wading along a steep margin such as a highway embankment, loosening the soil above the water's edge with the weeder, picking out those larvae showing up, and then exposing others by washing a double handful of water up onto the loosened dirt. Deerfly larvae may be kept together in the same container when collected, but more than two or three Tabanus larvae together are unsafe because of the restless and rapacious nature of these large larvae. Immediate separation is necessary after reaching the laboratory. It was found convenient before leaving the station, to prepare a number of vials in a partitioned box as described earlier, in which case, the larvae were easily separated in the field and were then ready for immediate serial treatment on return without the necessity of further transfer.

It is interesting to note that with Cameron, the abundance of larvae of a particular species was proportionate to the number of adults in any particular neighborhood. Under Minnesota conditions the reverse was true; the most abundant larvae represented the least noticeable adults in most cases. *Tabanus trimaculatus* Pal., *Tabanus reinwardtii* Wied., *Tabanus nivosus* O. S. and *Tabanus stygius* Say were all abundant as larvae but seldom met with as adults and of infrequent occurrence in collections. It is probably true with us, as suggested by Jones and Bradley (1923) for Louisiana species, that considerably more data will have to be gathered on terrestrial species before we will know the whole story concerning Minnesota tabanids.

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PRELIMINARY REPORT ON OBSERVATIONS ON THE DEVELOPMENT OF OVA OF PIG AND HUMAN ASCARIS UNDER NATURAL CONDITIONS, AND STUDIES OF FACTORS INFLUENCING DEVELOPMENT *

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and
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State laboratory reports of Alabama since 1924 show a very low infestation with Ascaris among the general population, it being less than 0.2 per cent in the lower coastal plain and approximating 3 per cent in the northern counties. Special surveys (Smillie and Augustine; Caldwell) tend to corroborate these findings. Surveys in Florida (Kerr, 1926) and the findings of the Rockefeller Sanitary Commission (1914) and the state laboratories of Georgia and South Carolina likewise indicate a low Ascaris infestation in those states. On the other hand, ascariasis constitutes a public health problem in Tennessee, North Carolina, Kentucky, and Virginia, being most prevalent in the mountain regions. In both south Alabama and west North Carolina, however, infestation among pigs approaches 50 per cent (Caldwell). In search for an explanation of this distribution, various observations were made in 1926 and 1927.

To make these studies possible it was necessary to isolate ova from soils. Briefly, the method devised makes use of (1) antiformin solution to release the ova, (2) sugar solution of high specific gravity to float up the ova, and (3) a small vial to remove them from the surface. Differential counts of the varying stages of development—undeveloped, morula (early, moderate, and late), tadpole, motile, and disintegrated—in 200 to 500 ova give in a sense a quantitatively accurate picture of conditions.

PART I

The first phase of our study considered the relation of types of soil and the influence of varying seasonal conditions on the development of ova in South Alabama. The soils, analyzed by the Bureau of Soils, included the nine main types of sands, loams, silts, and clays. Humus from the Florida Everglades was included. Ten cultures each were made. We mixed 5 grams of feces, pig or human, in 25 grams of soil, incubated, moistened daily, kept as nearly as possible under equal condi-

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tions, and isolated in 14 to 16 days. An analysis of results tend to indicate that under otherwise equal conditions, one soil is approximately as good as another as culture media for the development of Ascaris ova.

Equal amounts of feces, pig or human, were also "planted" on top of these soils out of doors in wire baskets sunk into the earth under a mulberry tree, and left under natural conditions, protected only from animals by netting. Plants on three types—sand, loam, and clay—were made in August of 1926, and on the remaining soils late in November and early in December. Plants were also made on the sandy soil of the laboratory yard in sun and shade in the summer and late autumn. These were examined at intervals, and records were kept of the maximum and minimum temperatures and precipitation.

As Brown noted in Panama (1926), we also found that in summer ova disintegrated in feces in the sun, those in the human feces more rapidly than those in the pig, while in the shade practically all ova developed in 15 to 19 days, the majority being in the motile stage. Ova in feces in the sun from the middle of November to early spring did not disintegrate. From late November to late January, practically no development occurred in ova in human feces, only 3 per cent being in the early morula stage, but 93 per cent of the ova in pig feces were in the morula stage, 50 per cent in the more advanced form of this stage. During this period, though the temperature had fallen below freezing eleven times, the maximum disintegration was 5 per cent. On January 21 no feces were visible in the August plants, but motile ova were readily recovered from the clay soils tested.

By February 11, with an increase in temperature, but also after 11 days of continuous dry weather, the processes both of development and disintegration were apparent. The ova in pig feces now contained motile embryos or were in advanced states of development, while human types were not developed beyond the morula stage, the majority being still undeveloped or in the early morula stage. Disintegration ranged from 4 to 56 per cent. Up to this time the fecal specimens had remained more or less compact. Now forces other than temperature and humidity become important: insects in breaking up, removing, and mixing the feces with the soil; and plant life in conserving moisture and providing shade. When examined again in early April and late May, only small portions of crusty feces remained or they had entirely disappeared. The pig feces remained compact somewhat longer than the human. In early April, ova in plants of pig feces were either motile or disintegrated, and in late May, 50 per cent of ova in some feces were disintegrated, while 80 to 98 per cent in soils and in feces protected by moss were motile. In human feces in early April, 45 to 90 per cent of ova in feces, and 5 to 16 per cent in soils, were disintegrated. The viable ova were practically all developed, 5 to 28 per cent were motile, the highest percentage in soils. By the end

of May only two fragments of human feces remained, with practically all ova disintegrated in the one, and 45 per cent in the other, which was protected by leaves and moss. Ova in the soils were in motile or tadpole stages; disintegration ranged from 20 to 50 per cent. In plants of both pig and human feces, ova were recovered with difficulty in some instances. Ova in numbers were readily isolated from both the sand and clay soils of the plants made nine months previously, more than 95 per cent actively motile.

PART II

The observations made during the year suggested further concrete studies on the influence of sunlight, heat, drying, moisture, low temperatures, and individual variations. Thus far studies have been carried forward in part on the first four factors, which are somewhat interdependent. To rule out individual variations, the mixed feces of several persons or pigs, were planted in equal volume on sand, sandy-loam-humus, and clay, and cultures also were employed in some experiments.

Development in Shade: For controls, two series, A and B, were planted July 2 and 8 in shade under natural conditions. All the pig feces remained compact; the human in Series A were attached by ants and spread somewhat thinly by rains. In both series the ova in the pig feces developed more rapidly and uniformly than those in the human feces. In Series B in 10 days, 94 per cent of the ova in the pig feces and 46 per cent of those in the human feces were developed; in the pig feces a few were already motile. In 24 days, 90 per cent of the ova in the pig feces and 33 per cent of those in human feces were motile, with 16 to 24 per cent of latter undeveloped. When last examined August 19, 99 per cent in the pig and 72 to 92 per cent in the human feces in both series were motile. The longest period without rain was six days.

Drying in Sunlight: With this development, we may compare the effect of drying on the same series placed in sunlight. The maximum temperature of the air in shade during this period was 98 degrees. Although the temperature of the dry soils varied considerably at times, the temperature of the fecal plants differed but little from each other. The maximum temperatures noted were respectively 126°, 138°, and 146° F. on clay, sand, and sandy-loam-humus, when the temperatures of the feces was 130° F. After exposure for three days, two and one-half of which were clear, all ova in Series A were completely disintegrated, as were the ova in five sets of sand cultures. The exact hours of sunlight were noted in Series B. After 7½ hours the feces had dried to thin crusts, but were moist within, and 35 to 50 per cent of the ova in the human feces, and 5 to 30 per cent of those in the pig were disintegrated, the highest percentages in sandy-humus. After 15 hours, all fecal plants were dry throughout, and all ova degenerated. The actual number of days exposed was again three.

Motile embryos in sandy cultures, tested on two different days at the same time, became hyaline in 3½ hours, while in cultures of the same series kept moist, 99 per cent remained actively motile. Cultures of undeveloped ova in all the soils indicated were tested in late July. Light clouds intermittently obscured the sun and the maximum temperature of the soil did not exceed 106° F. After 7 hours, complete degeneration of Ascaris ova ranged from 50 to 80 per cent, and of Trichuris ova, from 70 to 100. All ova were hyaline in an additional three hours in the sun the next day, when the maximum temperature was again 106° in soil.

Drying in Shade: Series A and B were at the same time placed under the laboratory building, where the feces dried but were otherwise under natural conditions. Disintegration was not apparent within 10 days, when practically all ova in the pig feces were in advanced states of development, and approximately 20 per cent of those in human feces showed development, the majority being in the early morula stage. In 16 days the effect of drying was evidenced by marked fading, shrinking, and collapse, though few were completely hyaline. In Series B development was somewhat more rapid and evidence of disintegration slightly less than in Series A. The ova in the pig feces were either motile or tadpole, with 14 to 38 per cent disintegrated. In the human feces none were motile, and 26 to 36 per cent were disintegrated. In the course of one and two weeks respectively, in the two series, the majority of ova were hyaline and the viability of others questionable. The movement of embryos still viable was very sluggish. In Series B, a few ova in human feces had developed to the motile stage before disintegration. It was not, however, until 15 days later that all ova were hyaline. In a third series, C, noted late in August and early in September, when temperatures ranged to 100° F. and no rain fell, the effect of drying was apparent in 14 days in a retardation of development and increase in disintegration, and by the end of the third week, it was very doubtful whether any ova were viable. No ova developed to the motile state in either the pig or human feces, the majority of the latter showing no development before degenerating. Trichuris ova showed marked effects of drying the first week, and in 14 days probably none were viable.

In cultures, Series C, though in 6 days they appeared dust dry, the maximum disintegration was 3.5 per cent, and 90 per cent of the ova in pig feces and 75 per cent of those in human feces were developed to the moderate morula stage; 90 per cent of Trichuris ova were degenerated. In 13 days, 75 per cent of pig and 38 per cent of the human feces contained motile embryos, but the effect of drying was now evident in the shrinking of embryos. From 2 to 8 per cent of the Ascaris and all Trichuris ova were hyaline. In another week, degeneration was pronounced, in clay and loam cultures slightly less marked. During the period of these observations, begun July 26, a large proportion of the

days were partly cloudy. In a similar series, of August 11, ova developed to the tadpole and advanced morula in 7 days, but after a week of extremely dry weather, though not hyaline, the ova were so shrunken that accurate differential counts could not be obtained.

Drying in Incubator: Series A and B were placed in incubator, with temperatures in the daytime ranging from 40 to 53 degrees C., and room temperatures at night. The highest temperature in the incubator differs but little from the maximum noted in feces in sunlight, and the high temperatures were better sustained. The effect of drying became noticeable in the pig in 3 days time, when one-third of the ova showed fading and shrinking, and by the end of 96 hours (33 hours of high daytime heat) all ova were hyaline, while only 10 per cent of the human type had degenerated. It was a full week, 70 hours of high temperatures, before the human feces were completely dry and all ova hyaline. Under continuous temperatures, 40-50 C., in Series C (including also loam), the ova in pig feces showed the effect of drying in 48 hours and were completely degenerated in 72 hours; the human became degenerated 24 hours later. In cultures at this temperature, ova were faded and shrunken at the end of eight hours, and approximately 95 per cent of all ova were hyaline in 11 hours. In temperatures 42-48° C. except in the clay cultures, 90 per cent of the ova had degenerated in 10 hours. In clay complete disintegration was slightly retarded.

Moist in Sun: The fecal plants of Series A and B kept moist in the sun by hourly sprinkling remained intact except for crumbling due to sampling. Moss began to grow in the baskets of Series A. In both series, the ova of the pig feces showed little disintegration—maximum 7 per cent—and though their development lagged two weeks behind those in the shade, in 30 days 85 per cent were developed, 35 per cent motile. A week latter 63 per cent were motile. Though in the human feces ova show a tendency to disintegrate without development, degeneration is not rapid. In Series B, in 30 days 11 per cent had developed, of which 3 per cent were motile, and 49 per cent had degenerated. In Series A, protected somewhat by moss, 27 per cent had developed and 23 per cent were disintegrated.

Moist in Incubator: In the fecal plants of the same series kept moist in the incubator under intermittent high heat, as noted previously, the development of ova in pig feces in the first 10 days approximated that under natural shade conditions, but thereafter development was retarded and a tendency to disintegrate was noticeable. At the end of the month development slightly exceeded that of the same feces in the sun, and 10 per cent were degenerated. In the human feces only occasional ova showed development, and in 30 days but 5 per cent were developed and degeneration was pronounced in 40 per cent. When kept moist under

constant temperatures of 40-50° C., no development occurred in the human feces in 14 days and an average of 38 per cent of the ova were disintegrated. Degeneration in ova in pig feces was practically nil, and though development was greatly retarded, in two weeks 47 per cent were in the morula stage, 17 per cent beyond the early phase. When transferred to room temperature, 85 per cent were motile in 13 days. The ova in human feces failed to develop and 50 per cent were disintegrated.

Varying Moistures: In North Carolina in feces planted on the bank of a stream under supposedly ideal conditions of moisture, ova failed to develop in 22 days, while 85 per cent of ova in feces on rocky soil in shade developed. This fact, together with variations observed in our experiments, suggested that degrees of moisture might influence development. Three sets of cultures, pig or human feces, were made in duplicate in sandy-loam, moistened with 1 cc. of water (1) daily, (2) alternate days, (3) every fourth day. A fourth set were kept just saturated, stirred every other day for aeration. These were kept at room temperatures. Despite the fact that to the eye the cultures of sets 2 and 3 seemed dry, within 15 days 89 to 97 per cent of the ova in pig feces in the first three sets and 57 to 62 per cent of those in the human feces contained motile embryos. Though the rates of development were approximately equal, that in sets 2 and 3 was perhaps slightly more rapid. In the saturated cultures only 2 per cent of the ova of the human feces and 36 per cent of those of the pig were in the *early morula stage.* It was not until 3 and 4 weeks later that the development in saturated cultures approached the development cited in the first three sets, and the development in the human feces did not closely approximate that in the pig until 2 weeks later, when 86 per cent were motile and 7.5 per cent degenerate. In a repetition of the saturated series, retardation was even more marked, owing in part to a slight lowering in temperatures.

GENERAL DISCUSSION

From the observations and experiments cited, it is clear that ova in pig feces develop (1) under lower temperatures, (2) under higher temperatures, and (3) under normal summer temperatures more rapidly than the human type. They also suggest, however, that in part this difference has its explanation in the character of the feces, since in cultures the rates of development more closely approximate each other. Though aeration and variations in acidity and bacterial decomposition may play a part, it is also highly probable that the moisture conditions in pig feces more nearly approach optimum for development. But even under the most favorable cultural conditions the development of ova in pig feces is more uniform and its rate of development is somewhat greater than that of ova in human feces. In a comparison of numbers of individual specimens under conditions optimum for ova in human feces, the rates

of development may over-lap, but our studies would suggest that they differ under varying conditions. The character of the human feces in winter in which the ova did not develop, was not different from the feces in summer in which development was fairly rapid, so that in contrast the development of the ova in the pig feces in the low temperatures of the winter months is significant. The ova in the pig feces, moreover, begin to develop under higher temperatures and are more resistant to the ill effect of long continued heat. These variations suggest a biological difference between them.

Desiccation is the greatest lethal factor to the development of Ascaris ova. Hence forces which tend either to hasten or retard drying—temperature, humidity, plant and insect life—are of importance in the epidemiology of ascariasis, where soil pollution is present. The high temperatures in the sun in the Tropics and in the summer heat of temperate zones, may be injurious, but this factor is probably offset by favorable conditions for development in the shade. Though drying is somewhat retarded in clay soils, and in the sun sandy soils reflect higher heat, it is not probable that these differences play a major part in the epidemiology of ascariasis.

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EXPERIMENTAL TRANSMISSION OF TRICHOMONADS FROM THE INTESTINE AND VAGINA OF MONKEYS TO THE VAGINA OF MONKEYS (*MACACUS RHESUS*) *

Robert Hegner

Trichomonads have been described from the intestine and vagina of both human beings and monkeys. The species that lives in the human intestine is known as *Trichomonas hominis;* this form occurs with three, four or five anterior flagella and may actually represent three species or varieties. It is distributed throughout the world and is present in a considerable percentage of the general population. The species known as *Trichomonas vaginalis* has been reported from the vagina of from 10 to 50% of women from various countries and has also been found in the urinary tract of man. *Trichomonas hominis* and *Trichomonas vaginalis* are usually considered to be distinct species although there is still some question of this. Trichomonads have been described from the intestine of at least six species of monkeys including the chimpanzee and the orang-utan. A small species from the chimpanzee was given the name *Trichomonas anthropopitheci* by Deschiens (1927). Trichomonads have also been found in the vagina of monkeys (*Macacus rhesus*) and have been named *Trichomonas macacovaginae* (Hegner and Ratcliffe, 1927).

Two problems of interest and importance involve these intestinal and vaginal trichomonads: (1) do the vaginal and intestinal trichomonads of man belong to one species? do the vaginal and intestinal trichomonads of the monkey belong to the same species? are those of man and monkey really specifically distinct? and (2) how are vaginal trichomonads transmitted? The experiments described below were carried out in order to aid in the solution of these problems. The writer is greatly indebted to Dr. Carl G. Hartman of the Department of Embryology of the Carnegie Institution of Washington, located at the Johns Hopkins University, for assistance in obtaining material and for the use of monkeys belonging to his colony. The difficulties involved in work of this type were found to be very great, so great indeed that the data obtained are published herewith in preliminary form since more satisfactory material must be available to make further work of any great value.

* From the Department of Protozoology, Johns Hopkins School of Hygiene and Public Health. This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

Six female monkeys of the species *Macacus rhesus* were used in these experiments and cultures of trichomonads obtained from the intestine and vagina of the same species of monkey. These trichomonads were cultivated in a medium consisting of 0.7 gram NaCl and 1 gram NaCit in 100 cc. of distilled water to which was added 0.5 gram of Loeffler's dehydrated blood serum. All cultures were incubated at a temperature of about 36°C. Smears and cultures were made from washings of the vagina of the six monkeys used. Washings were examined six times during the two weeks preceding the experiments and on the date of the first experiment (May 12) and were all found to be negative.

On May 12, 1.5 cc. of culture material containing many trichomonads from the intestine of the monkey was injected into the vagina of each of the experimental monkeys Nos. 29, 30, 31, 32, 34 and 39. Washings from these six monkeys were examined on the dates given in Table 1. On the second day after inoculation (May 14) washings from the vagina of the six monkeys contained trichomonads, except from No. 32. Examinations on later dates, as shown in the table, indicate that persistent infections were set up in monkeys No. 29 and 30 but that monkeys No. 31, 32, 34 and 39 did not become infected. The trichomonads found in monkeys No. 31, 34 and 39 on May 14 had evidently lived in the vagina for two days but were unable to set up an infection.

Having apparently set up infections in the vagina of monkeys 29 and 30 with intestinal trichomonads from the monkey, it was decided to inject trichomonads from the vagina of an infected monkey from the colony into the vagina of monkeys 31, 32, 34 and 39 which had resisted infection with the intestinal trichomonads. On June 6, 1.5 cc. of culture material containing numerous vaginal trichomonads was injected into the vagina of these four monkeys. The results of later examinations are shown in Table 2. It appears that monkey No. 31 became infected but that the other three resisted infection even with vaginal trichomonads although the finding of a trichomonad in the vaginal washing from monkey No. 39 on June 13 suggests that there may have been a very light infection in this animal.

The data presented in Table 1 indicate that trichomonads from the intestine of *Macacus rhesus* are capable of setting up an infection in the vagina of this species of monkey. Failure to set up infections in monkeys 32, 34, and 39, as shown in Table 2, with trichomonads from the vagina of *Macacus rhesus* indicates that those monkeys were naturally resistant to trichomonads that normally live in the vagina. The evidence from these experiments favors the hypothesis that the trichomonads from the intestine and vagina of the monkey belong to one species. The writer has been unable to detect any morphological differences between the trichomonads from the intestine and vagina of

these monkeys. Those who have worked with monkeys know how easy fecal material contaminated with trichomonads may reach the vagina. This offers a simple explanation of the transmission of these flagellates, that is, a monkey acquires an intestinal infection by the ingestion of food or drink contaminated with trichomonads and then the vagina becomes infected by contamination with trichomonads passed in the feces. It is not known whether trichomonads are able to live in the urinary tract of male monkeys. If they are, they might reach this location during coitus with an infected female.

Table 1.—*Table Presenting the Results of Examinations of Vaginal Washings from Four Monkeys (Macacus rhesus) into the Vagina of Which Culture Material Containing Trichomonads from the Intestine of a Rhesus Monkey was Injected on May 12*

	29*	30†	31	32	34	39
May 14	—	+	+	—	—	+
May 17	+	—	[illegible]	—	—	
May 19	—	—	—	—		
May 21	—	—	—	—		—
May 25	—	—	—	—		
May 27	—	—	—	—		
May 31		—	—	—		
June 2		+	—	—		

* Smears from no. 29 were positive on May 14, 17, 27. No. 29 was positive also on June 17 and June 23.
† Smears from no. 30 were positive on May 27, June 2. No. 30 was positive also on June 13.

Table 2.—*Table Presenting the Results of Examinations of Vaginal Washings from Four Monkeys (Macacus rhesus) into the Vagina of Which Culture Material Containing Trichomonads (T. macacovaginae) from the Vagina of a Rhesus Monkey was Injected on June 6*

	31	32	34	39
June 8	+	—	—	—
June 10	+	—	—	—
June 13	+	—	—	+
June 16	+	—		—
June 23	+	—	—	—

Human vaginal infections may be brought about in a similar way provided the intestinal and vaginal forms of man belong to the same species or are able to live in both the intestinal and vaginal habitats.

SUMMARY

Trichomonads from the intestine of the monkey, *Macacus rhesus*, were grown in serum-saline-citrate culture medium and injected into the vagina of six monkeys of the same species that had previously been found to be free from these organisms. Two of the six monkeys became infected.

Trichomonads from the vagina of a monkey, *Macacus rhesus*, were also grown in serum-saline-citrate medium and injected into the vagina

of the four monkeys that did not become infected by the intestinal trichomonads. One of the four became infected; the other three resisted the infection.

Previous studies indicate that the trichomonads of the intestine and vagina of the monkey, *Macacus rhesus,* are morphologically alike. The results of these transmission experiments favor the hypothesis that these two types of trichomonads belong to the same species. It is suggested that vaginal infections in the monkey arise from the entrance into the vagina of fecal material containing trichomonads.

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A NEW NEMATODE PARASITE, *TETRAMERES PAUCISPINA,* FROM A SOUTH AMERICAN BIRD, *AMBLYRAMPHUS HOLOCERICEUS*

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The material here described was very kindly given to the writer for study by Professor E. E. Tyzzer of the Department of Comparative Pathology of the Harvard Medical School, who had secured and made preliminary observations on the parasite while performing a postmortem examination on a South American "black-bird" which had died in the Franklin Park Zoological Garden of the City of Boston. I am indebted to Dr. Tyzzer, not only for the material but also for his generous assistance in interpreting the anatomical changes in the host following the invasion by the parasite.

The material consisted of the proventriculus showing seven spherical cysts, each a little smaller than a pea, projecting outward through the proventricular wall into the peritoneal cavity. The cysts were contiguous with each other forming a cluster which, after dissecting the enclosed worm from two of the cysts, was preserved in formalin with the part of the proventriculus affected. One of the worms removed was fixed in Zenker's fluid and the other was flattened and cleared for microscopic examination. Macroscopically, the internal aspect of the wall of the proventriculus appeared normal; nor were there, aside from minor changes involving a few of the tubulo-sacular, "chief" glands lining this organ in the immediate vicinity of the cysts, any marked pathological lesions to be noted.

The female is globular in shape, measuring about 2.38 mm. in diameter and is marked externally by four shallow furrows lying in the dorsal, ventral and lateral fields of the body which is thus divided into four quadrants (Fig. 1). The worm presents an annulated surface. The true striations of the cuticula, however, are only visible under high magnification. In the fixed material in situ within the cyst, the filiform cephalic and caudal extremities of the female are not visible, but in the living specimen these parts project outward from the globular body to the extent of about 0.25 mm. and are also notable features in the fixed dissected specimen.

Two slightly imperfect sets of serial sections of isolated cysts were cut 8μ thick but from this material it is, unfortunately, not possible to build up any structural details of the female genital organs which would be sufficiently reliable to warrant description in this paper. The body is so distended with the numerous coils of the ovaries and uteri that the

alimentary tract and posterior vaginal region of the genital system is subjected to a distorting pressure.

The egg (Fig. 6) is ellipsoidal in outline and measures from 49.5μ to 51.7μ in length and 30.1 to 38.7μ in breadth. Its wall has considerable thickness and at each pole it is adorned with a spangle of filamentous threads. These threads are not present in the young eggs in the upper passages of the uterus but are only laid down as the egg develops and passes down the uterus toward the outside. The egg when discharged contains a fully developed embryo. In sections, eggs are sometimes found embedded, here and there, in the glandular lining of the cyst but they are probably discharged directly into the lumen of the proventriculus through the aperture of sacculo-tubular gland, although the caudal extremity of the female was not found protruding from its cyst.

On carefully dissecting two of the preserved cysts under a low-powered microscope, a single male worm was found lying on the surface of the female. Removal of the male worm leaves a well-defined mould of its body impressed on the glandular lining of the cyst. Heretofore, the male of various species of the genus Tetrameres has been described as living free in the lumen of the proventriculus, and indeed Dr. Tyzzer found two male individuals in this location. The association of the male with the female inside the cyst is a novel observation and it appears probable that fertilization of the female takes place within the cyst after which the male either perishes or escapes from the cyst for no trace of a male worm was found in either of two cysts which were serially sectioned for purposes of study. The residence of the males within the cyst may possibly account for the failure of several investigators to find males and they have thus been forced to describe species of this genus based only on the basis of the characters of the female.

As has already been stated, a fully mature male was found associated with the globular female in each of the two cysts that were dissected. In addition to this, two males were found free in the lumen of the proventriculus. Thus four males were available for study and the following description is based on this material. The total length ranges from 3.1 to 4.5 mm., the maximum thickness being ca. 0.185 mm. The worms are robust in their build, tapering obtusely at the head end and posteriorly forming a conoid tail. The annular striations in the cuticula are well-marked, the striae being about 4 microns apart. The mouth is surrounded by three minute lips, radially disposed. The buccal cavity is infundibuliform and only 9μ deep. The pharynx is 0.120 mm. long and is crossed at its base by the nerve ring. It is followed by the esophagus, 0.370 mm. long. Cervical papillae are present but they are small and very inconspicuous. They are situated about 100μ from the anterior extremity of the body, some distance in front of the nerve ring. The excretory pore is too small to be located in any of the preserved specimens.

At first appearing to be totally devoid of spines, a close scrutiny of the properly cleared worms under the highest magnification of the microscope, discloses a single series of minute, transparent, conical spines, 6.8μ in length adorning the median ventral field. These spines extend only through the posterior two-thirds of the body and are not more than 25 in number, of which three are post-anal. The cloaca is about 94μ from the caudal extremity. The testis extends almost as far forward as the anterior end of the intestine at which point it is recurrent for a short distance. In series with the testis is a pyriform seminal vesicle opening into the cloaca. The two spicules are dissimilar in size and shape. They are both exceedingly slender. The larger spicule varies in size from 0.328 mm. to 0.371 mm. and is about 0.009 mm. thick. Sometimes lying closely apposed to this spicule, and hardly discernable because of its relatively weak chitinization is the second spicule. In two favorable specimens, the second spicule was found to measure 0.012 mm. and 0.154 mm. long, respectively.

The reaction of the host tissue to the presence of the parasite is of interest. Since the cyst containing the worms has a glandular lining, it is evident that the female establishes itself early in life in one of the crypts into which the "chief" glands of the mucosa open. As the parasite grows to maturity, it bulges outward, pressing through the muscularis mucosae and the thick middle or circular muscle layer and stretching the outer longitudinal layer so that this is scarcely demonstrable on the outer aspect of the cyst. It is difficult to conjecture how this is accomplished for the firm muscular layer would supposedly furnish more resistance to the pressure of the growing parasite than would the soft mucosa. The glandular lining of the cyst stains rather differently from the normal chief glands but in serial sections it is found to be continuous with the epithelium of the sacculo-tubular gland from which it appears to be an outgrowth, for the integrity of the greater portion of the latter is preserved. There being direct communication between the cyst and the glandular sac, it appears probable that the female worm extends its caudal extremity carrying the vulva, inward through the aperture of the gland in order to discharge its eggs into the cavity of the proventriculus. The passage from the glandular sac is lined with epithelium and may contain a black fecal material derived from the worm, as well as occasional ova. Around the orifice in the surface of the mucosa, there are collections of leucocytes, and other evidences of inflammation indicating the injurious effect of the presence of the parasite (Fig. 2).

Reference to the admirable monograph of Cram (1927) on the nematode parasites of birds, and comparison with the more important original descriptions show that the form here dealt with is distinctly different from any species in the genus heretofore described. In the smallness of the male and the corresponding brevity of the spicules, the

organism resembles *T. nouvelli* Seurat, and *T. fissispina* (Diesing) Travassos; but it is easily distinguishable from these species as well as from other species of the genus by the arrangement of the cuticular spines as well as by the disproportion of the several parts of the alimentary tract as compared with species already described. In addition, the present host is a new one for the genus. In view of these considerations, it is believed that the parasite is new to science, and for it the name *Tetrameres paucispina* is proposed. The type species is to be deposited in the helminthological collection of the Museum of Comparative Zoology of Harvard University.

Reference Cited

Cram, Eloise B. 1927.—Bird Parasites of the Nematode Sub-orders Strongylata, Ascaridata and Spirurata. U. S. Nat. Museum. Bull. 140, 465 pp., 444 figs.

Explanation of Plates XII and XIII

All figures were drawn with Leitz microscope and with the aid of the camera lucida. The size of the organism is indicated by the scale accompanying each figure.

Plate XII

Fig. 1.—Female dissected from a cyst showing longitudinal furrows and the transverse annulations.

Fig. 2.—Diagram of serial section showing the female in situ within the cyst and indicating the relationship of the parasite to the wall of the proventriculus.

1

2

PLATE XII

Plate XIII

Figure 3.—The male showing general organization and distribution of the cuticular spines.

Fig. 4.—Anterior extremity of the male to show the location of cervical papillae and the parts of the alimentary tract.

Fig. 5.—Posterior extremity of the male showing spicules, etc.

Fig. 6.—Mature egg with polar filaments.

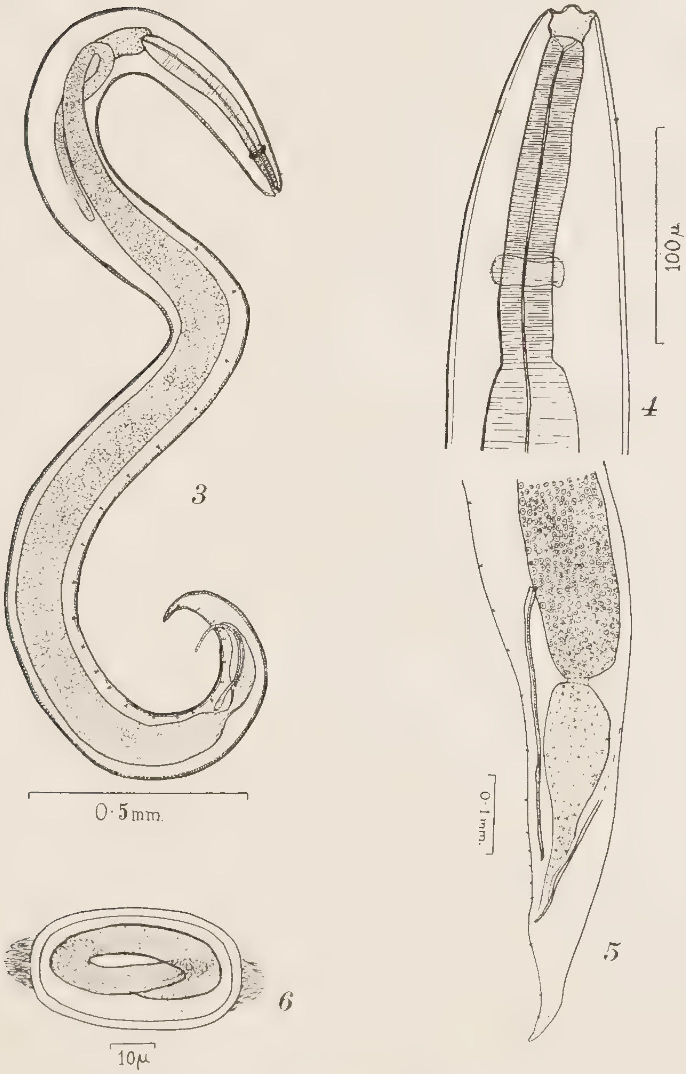

PLATE XIII

MEGALOGONIA ICTALURI, A NEW SPECIES OF TREMATODE FROM THE CHANNEL CATFISH, *ICTALURUS PUNCTATUS*

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In the course of studies on the trematode parasites of Minnesota fishes, I have several times noted a form in the Channel Catfish (*Ictalurus punctatus*) which presents some very characteristic features. In the structure of the testes it represents not only a new species but an undescribed genus.

The Channel Catfish in which these specimens were found were taken from the St. Croix River at Stillwater, Minnesota, on January 10, and from the Mississippi River at St. Paul, Minnesota, during its high-water stage on April 30 and again on June 22, 1927. In five out of sixteen of the fish, the minute flukes described below were found in folds of the intestinal mucosa. They were not found in any particular region, but were more abundant in the lower intestine where they were usually found with large numbers of a small Allocreadian species. Specimens of this species were obtained by stripping the mucosa and placing it in petri dishes in water, where the flukes soon betray their presence by their activity in freeing themselves from the mucosa.

The adult distome is somewhat depressed, pear-shaped, with its greatest width near the anterior end of the testes. The neck region is mobile and translucent white in the living specimens, while the region posterior to the acetabulum is white and mostly opaque due to the presence of massive testes which nearly fill the posterior region. Eleven specimens were found to average 0.753 mm. in length and 0.340 mm. in width. The range in length for these specimens was from 0.404 to 1.434 mm. The range in width was 0.246 to 0.580 mm.

Two well-developed suckers are present. The oral sucker at the anterior end of the body is ventral in position and in eight specimens averaged 0.107 mm. in transsection. Slightly anterior to and dorso-lateral to the middle of the oral sucker are two papillae, one on each side. They measure about 25μ in width by 21μ at the base in the extended condition. These papillae are scarcely noticeable in most fixed specimens and their presence might have been overlooked but for observation of the living specimens in which the hornlike papillae were demonstrated as the animal groped about in moving forward. The acetabulum is from one-fourth to one-third of the distance from the anterior to the posterior end of the body. In eight specimens it measured on the average 0.127 mm. in transsection, with a range of 0.102 to 0.176 mm. The

ratio of the diameters of the oral and ventral suckers is 1 : 1.18. Upon the surfaces of the two suckers were noted minute spinous processes, apparently indefinite in arrangement. The integument is otherwise without spines or prominences.

The mouth in the oral sucker leads directly into a well-developed, barrel-shaped pharynx 86.8μ long and 82.9μ in transsection. A short oesophagus is present behind which the digestive tube bifurcates and runs caudad to a position near the posterior end of the worm. The intestinal ceca are about 21μ in diameter and are slightly enlarged at their posterior extremities. A relatively large, simple sacculate excretory bladder is present. The excretory pore is terminal in position.

The most characteristic feature of the worm is presented by two pairs of proximate, lateral, massive, slightly-lobed organs, which are pyramidal in general shape in fixed specimens. They almost fill the post-acetabular region of the body. In a specimen 0.572 mm. in length, the anterior testis on the left side was found to be 0.114 mm. long and 0.070 mm. wide. The posterior testis, lying immediately caudad, is 0.108 mm. in length and 0.064 mm. in width. The anterior testis of this side is at a distance of 0.284 mm. from the anterior end, while the posterior extremity of the posterior testis is 0.071 mm. from the posterior end of the worm. The anterior testis of the right or ovary side is 0.316 mm. from the anterior end, slightly more posterior in position probably due to the presence of a large, pear-shaped ovary and receptaculum seminis. This testis is 0.106 mm. in length and 0.089 mm. in width. The posterior testis of this side is 0.047 mm. from the posterior end of the worm. Both pairs of anterior and posterior testes are slightly lobed about their transmedian line. Several specimens were sectioned longitudinally before it was conclusively demonstrated that not one pair but two pairs of testes are present. The testes in a living specimen of this fluke were observed as closely as the activity of the worm permitted. In the living condition, the two pairs of testes are so proximate in position that they do not appear to be two distinct pairs of organs, as they were actually found to be. Instead, they seemed to occur as two massive, lobed organs opposite each other and nearly filling the posterior region of the body.

A very well-developed cirrus sac is present. It is cresentic in form in living specimens and contains the cirrus, seminal vesicle, ductus ejaculatorius and prostate glands. In a fixed specimen with cresentic cirrus (a specimen somewhat below the average in size—0.404 mm. in length), the cirrus sac was found to be about 0.214 mm. in length and 0.075 mm. in maximum diameter. Its posterior extremity is posterior to the acetabulum and near the origin of the oviduct. From this point it arches dorsally over the acetabulum to the median genital pore near the

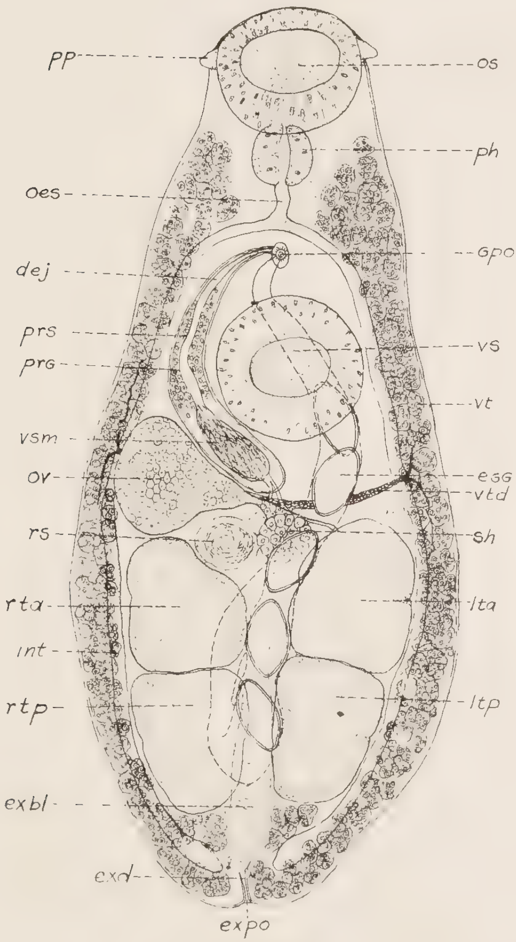

PLATE XIV

Description of text figure. *Megalogonia ictaluri,* mature specimen (× 215). *dej,* ejaculatory duct; *exbl,* excretory bladder; *exd,* excretory duct; *expo,* excretory pore; *gpo,* genital opening; *int,* intestine; *lta,* left anterior testis; *ltp,* left posterior testis; *oes,* esophagus; *os,* oral sucker; *ov,* ovary; *ph,* pharynx; *pp,* papilla; *prg,* prostate gland; *prs,* prostate part of cirrus organ; *rs,* receptaculum seminis; *rta,* right anterior testis; *rtp,* right posterior testis; *vd,* vas deferens; *vs,* ventral sucker; *vsm,* seminal vesicle; *vt,* vitellaria; *vtd,* vitelline duct

bifurcation of the intestine and between it and the acetabulum. Prostate glands occur in the anterior half of the cirrus sac.

The female genitalia consist of ovary, oviduct, seminal receptacle, vitellaria and vitalline ducts, shell gland and uterus. The ovary lies just anterior to the anterior testis of the right side. It is pear-shaped and measures 0.066 by 0.077 mm. A short oviduct proceeds laterally to the ootype. On the right of the ootype is a pyriform receptaculum seminis which measures 27 by 34.2μ. Laurer's canal is absent. To the left of the receptaculum seminis is a group of about eighteen large cells, the shell gland. Extending lateral right and left from the ootype are the vitelline ducts which are concealed in most specimens of whole mounts by the confinement of ovary, receptaculum seminis, shell gland, testes, cirrus, etc., to a very small body space. The vitelline ducts are in a plane about midway between the anterior and posterior ends of the body. The vitellaria are lateral, well-developed, and extend from near the anterior end of the pharynx to the posterior end, becoming confluent behind the posterior testes. The yolk follicles occur in grape-like clusters and only partly cover the intestinal crura, being mostly extra-cecal.

The uterus is simple, usually proceeding caudad between the testes until near their posterior extremities when it describes a single loop ventrally and proceeds cephalad to the common genital aperture. Few ova were present in the uterus in most instances. An average of 5.7 with a range 2 to 15 eggs were found in the uterus of seven mature specimens. They are relatively very large and oval in shape, measuring 61.2 by 37.8μ. Eggs were observed both in living specimens and sectioned material without noting the presence of "plugs" or filaments.

THE SYSTEMATIC POSITION OF *Megalogonia ictaluri*

It has been quite impossible to place this distome parasite of the Channel Catfish in any family or generic group thus far recognized. It seems to have many characteristics in common with the Family *Allocreadidae* and the Subfamily *Allocreadiinae* (Odhner, 1905); among these are the single, undivided, sac-shaped excretory bladder, the median genital pore, the well-developed cirrus and cirrus sac, the lateral position of the ovary anterior to the testes, the few large eggs and extensive vitellaria which are confluent behind the testes. The nature of the testes in this species does not conform to the family or subfamily designation.

This preliminary note has been prepared for the purpose of calling attention to this unusual form. More detailed studies are under way and will be presented later. I am indebted to Professor William A. Riley, Head of the Department of Zoology, University of Minnesota, especially for direction and suggestions during the course of my work.

Specimens of *Megalogonia ictaluri* have been entered in the catalogue of the U. S. National Museum as follows: Type No. 7966; paratypes No. 7967.

THE CULTIVATION OF A PARASITIC AMOEBA FROM THE COCKROACH *

NANNIE M. SMITH

AND

HARVEY P. BARRET

The object of the present paper is to record the successful and long continued cultivation of a parasitic amoeba (*Endamoeba thomsoni*) from the cockroach, *Periplaneta americana*.

There seems no reason to review the previous work on the cultivation of the parasitic amoeba as this has been done very thoroughly in the recent paper of Dobell and Laidlaw (1926). It is to be noted, however, that the present work is a continuation of a series of investigations in which various parasites have been cultivated on a simple dilution of inactivated human serum in saline (*Blastocystis hominis* of man, Barret, 1921; *Balantidium coli* of man, Barret and Yarbrough, 1921; *Endamoeba barreti* of the turtle, Barret and Smith, 1924, and Taliaferro and Holmes, 1924; *Endamoeba ranarum* of the frog, Barret and Smith, 1926, and Taliaferro and Fisher, 1926). Although most of the cultivation of the parasitic amoebae of mammals has been done with the medium of Boeck and Drbohlav (1925) or with modifications of it, and although Dobell and Laidlaw (1926) were unable to use our medium for the cultivation of *E. histolytica* and several other amoebae of mammals, it is interesting to note that Craig (1926) has succeeded in growing *E. histolytica* in seven parts of 0.85% saline plus one part of inactivated human blood serum. This medium is obviously very similar to that used throughout our investigations and suggests that dilutions of inactivated serum in different percentages of saline may prove a very simple and efficient method for the cultivation of a large range of parasitic protozoa.

The original medium used by one of us (Barret 1921) in the cultivation of Blastocystis was a 1-10 dilution of inactivated human blood serum in 0.5 per cent salt solution. In the present work it was found best to double the dilution of serum (19 parts of 0.5 per cent NaCl solution and 1 part of inactivated human blood serum). In all of our work we have found 0.5 per cent salt solution to be superior to 0.7 or 0.85 per cent. This medium varied from p_H 7 to p_H 8 depending upon the length of time it was kept before use. In our experience a p_H anywhere in this range is satisfactory for the present amoeba.

All of the present work was done with the American cockroach, *Periplaneta americana*. The roaches around Charlotte, N. C., are almost

* From the Laboratory of Dr. Harvey P. Barret, Charlotte, N. C.

EXPLANATION OF PLATE XV

All drawings × about 2,500.

Figs. 1-3.—Three living specimens from culture.

Fig. 4.—A specimen stained with Heidenhain's iron hematoxylin

Fig. 5.—The same from a culture containing large amoebae.

invariably infected with amoebae, a fact which greatly aided in the cultivation of the parasites. The presence of numerous flagellates, blastocystis and yeasts, however, greatly handicapped the work. Thus, amoebae appeared in almost all of our early subcultures only to be overgrown with other organisms. We started approximately 36 lines of amoebae from as many insects, and although most of these were eventually lost by overgrowth of other organisms, two have now been maintained 24 months and are apparently capable of indefinite subculture.

The actual procedure of obtaining cultures from the insects was very simple. The broad lower end of the hind gut of each cockroach was dissected out and placed on a sterile slide. A few drops of the culture medium were added, the intestine macerated, and portions of the resulting mixture placed in the bottom of tubes of sterile culture medium. These inoculated tubes, as well as all of our first cultures, were placed in an ice box. Practically all of the cultures contained amoebae in five days. Each line was then transplanted at weekly intervals by transferring a small amount of the sediment to a fresh tube of culture medium with a capillary pipette. The two strains which survived the overgrowth of other organisms were transferred in this manner for three months. The poor growth of the cultures, however, suggested too frequent transfers and the interval was then extended to two weeks. At the end of another three months the transplants began to thrive poorly and they were placed at room temperature. The cultures at room temperature, transplanted at biweekly intervals, have grown well to date (April 1, 1927, a period of two years) and give every indication of the possibility of indefinite sub-culture.

A description of the amoeba is given by Dr. William H. Taliaferro in a note following the present paper.

CONCLUSIONS

1. An amoeba from the American cockroach *Periplaneta americana* has been successfully cultivated *in vitro* on a simple medium consisting of a 1-20 dilution of inactivated human blood serum in 0.5 per cent NaCl solution.

2. Cultures have been carried through successive transfers over a period of twenty-four months.

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A NOTE ON THE AMOEBA OF THE COCKROACH CULTIVATED BY SMITH AND BARRET

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Many of the cultures of the amoeba described by Smith and Barret in the preceding paper have been examined in this laboratory and I feel that the form is identical with that described by Lucas (1927) under the name *Entamoeba thomsoni.* Furthermore, this conclusion is corroborated by Miss Lucas who very kindly examined one of my prepared slides. In cultures, most of the living specimens were much vacuolated and filled with yeasts and other forms from the cultures (Fig. 1 and 2). At times, however, some of them exhibited very few vacuoles (Fig. 3), and in these the nucleus could be identified with certainty. At no time was a contractile vacuole visible. During locomotion the amoeba generally formed clear pseudopia composed entirely of ectoplasm although some specimens moved in a slug-like manner with very little differentiation of ectoplasm and endoplasm in the advancing pseudopodia. Many specimens exhibited a well marked "uroid," or finger-like projection at the end posterior in motion.

The size of the specimens varied greatly with different cultures. In general, on prepared slides, rounded specimens varied from 13 to 20μ in diameter. In a few cultures very large amoebae measuring up to 100μ were found which first were thought to be *Endamoeba blattae,* but nuclei in stained specimens (Fig. 4) indicated that they belonged to the *coli* group of *Endamoebae.* Many of these forms possessed two nuclei. They probably represent large forms of *E. thomsoni,* but I have no suggestion as to the factors underlying their appearance. Lucas gives the range of size for the living trophozoites as 16 to 64μ in length and 5 to 16μ in width.

The description of the nucleus of *E. thomsoni* by Lucas corresponds so exactly with the structure of the present form in stained preparations that there is no need for further description. A typical nucleus met with, showing the peripheral layer of chromatin, the karyosome and the intermediate area containing chromatin, is shown in figure 5. Other variations in structure have been noted as described by her. In many specimens the karyosome can not be identified with any certainty. In some cultures numerous cysts were seen and agreed with Lucas' description.

Paper Cited

Lucas, C. L. T. 1927.—Two new species of amoeba found in cockroaches; with notes on the cysts of *Nyctotherus ovalis* Leidy. Parasit., 19:223-235.

BOOK REVIEWS

The Life and Work of Sir Patrick Manson. By Philip H. Manson-Bahr and A. Alcock, with 12 half-tone plates, Cassell and Company, Ltd., London, Toronto, Melbourne and Sydney, 1927, 273 pp.

Among the outstanding figures of the past generation no one is more conspicuous in the field of medical zoology than Sir Patrick Manson. At the beginning of his career he worked unnoticed and amid unfavorable circumstances; that was the period in which he gained that breadth of knowledge and variety of experience which served him so well when at the age of 45 he settled in London and assumed at once by common accord a position of leadership that grew with every passing year. When he had begun his work little or nothing was known of the peculiar diseases of the tropics and their causes. His own numerous discoveries while quietly working as a government physician in the Orient were truly epoch making and started a long line of investigations which have built up the subject of tropical medicine as it stands today.

The volume under review has been prepared by two authors, one of whom, his son-in-law, Doctor Manson-Bahr, made full use of the diary and other personal material of Sir Patrick and is responsible for the main fabric of the book; the other author, Colonel Alcock, who was so closely associated with Manson in later years on the staff of the London School of Tropical Medicine which Manson founded and developed, has given us the story of Manson's work in China and of the mosquito-malaria hypothesis, one of the most striking features that work. The results of the author partnership are in conspicuous contrast with the usual outcome of such an arrangement for the volume is not only a unit but also an inspiring presentation of the theme which from cover to cover absorbs the interest of the reader and leaves him with the thought that the writers had with unusual skill painted the portrait of a great mind and a leader in the development of a vast field.

Patrick Manson was born on October 3, 1844, in Aberdeenshire but of Orcadian descent. As a child he was judged rather dull and as a school boy he was fonder of cricket than of books and was skilled in the art of hunting and fishing. One may regard as portentious the record that having shot a cat he became profoundly interested in the tapeworm he found while investigating its internal machinery in the seclusion of the housetop. He first took up mechanics but exceeded his strength and was compelled to give up active physical work, whereupon he exchanged engineering for medicine and studied both at Aberdeen and Edinburgh. He was able to pass his final examination before reaching the age of 20. Too young to be given a degree he went to London and spent his time in schools, hospitals and museums. He received his first degree in medicine in October, 1865, and on the basis of studies in comparative anatomy was given the M. D. in July, 1866. However, even before that he had secured an appointment as medical officer for Formosa to the Chinese Imperial Maritime Customs. He spent the next five years on that island inspecting the ships calling at that port, treating the crews and keeping the meteorological record. In addition to this official routine he had private practice among Europeans and Chinese and also served a missionary hospital. He traveled much even becoming acquainted with the aborigines of the mountains there and making many observations on items in his novel environment which proved of value later.

Early in 1871 Manson left Formosa rather abruptly and went to Amoy where he lived for 13 years. Here he made some of the most important discoveries on the origin of tropical disease as well as established relations with the Chinese which were of fundamental importance, for he succeeded in fairly disarming the native distrust of operations and after various successes had many opportunities

to use surgical procedure for the removal of the elephantoid tumors common there as in many other places in the tropics. Numerous interesting stories are cited, some from his own pen, to show the difficulties that surrounded him and his skill in extricating himself from the general distrust of western medicine current among the Chinese at that time. He succeeded in putting the missionary hospital on a better basis and establishing for it a reputation that was of prime importance. He was in truth a missionary of western medicine and developed this project in the same spirit that long afterwards was shown at the start of the School of Tropical Medicine in London. The authors comment rightly on the contrast between these modern days of traveling fellowships, of libraries and of research laboratories and the time when Manson in his isolation was thrown completely on himself for means of finding his material and developing it. A chapter is devoted to his surgical work in the treatment of elephantiasis, of liver abscesses and other then almost entirely unexplained conditions. From his notes it appears that in his work with the microscope he probably saw the bacilli of leprosy before they were originally described by Hansen.

In 1875 he returned to England and was married and worked some months in London at various hospitals. There he learned of the discovery of a microscopic nematode in the blood and urine of a patient who had suffered from chyluria. This discovery of Lewis made an impression on Manson and on his return occupied much of his time between 1875 and 1883. He predicted the location of the adults in the lymphatics and Bancroft verified this a year later. Lack of space forbids entering further on discussion of the story of this disease and its parasite, the details of which are clearly and judicially set forth in the book under consideration. This work attracted the attention of the distinguished English parasitologist Cobbold who accepted Manson's work without reserve and despite the disbelief of some other workers. Cobbold indeed was so much impressed by it that he did his best to secure a more favorable position for Manson, writing to various public officials, presenting reports of Manson's work before important meetings and everywhere urging the need of granting larger opportunities to one so experienced and so well fitted as Manson for the prosecution of research on tropical diseases.

In December 1883 Manson left Amoy and settled for private practice in Hongkong. His fame as a doctor preceded him and almost at once he was flooded with private practice that left little leisure and scientific work was necessarily neglected. Nevertheless he manifested his foresight and leadership in bringing about the organization of a local medical society, in the establishment of a dairy farm to supply pure milk for children and invalids and in his establishment of a school of medicine at a new memorial hospital which had been opened in that city. After five years of hard work he found himself in a position to retire. He had been in China for 23 years and now in March 1899 he left the Orient but only to place his knowledge of tropical diseases at the command of the world in a fashion that he certainly did not suspect. Unexpected financial difficulties compelled him at the summer of 1890 to open an office in London. It was for science a stroke of good fortune that practice did not come rapidly to this practically unknown consultant for he had an opportunity in a study in his own house to continue the research which had been started in China and through arrangements with various missionary societies to get especially from Africa blood films for examination. In 1892 he won an appointment as physician to the Seamen's Hospital Society and started at the Albert Dock Hospital a clinic and laboratory which soon became widely known. In 1894 he began to lecture on tropical diseases first to missionaries planning for tropical service. His reputation and influence grew rapidly. In 1897 he was appointed medical advisor to the Colonial Office. In 1898 appeared first his famous Manual of Tropical Diseases which during the next 20 years went through six editions, was reprinted many times and was translated into French and Spanish. Honors and privileges followed one another

rapidly now. In 1905 he came to America and by lectures and contacts gave an effective stimulus to the study of tropical medicine in the United States.

The Society of Tropical Medicine was founded in May 1907 with Manson as its first president. To his vigorous leadership was due in large part its remarkable growth in numbers and in influence. The London School of Tropical Medicine to which reference has already been made, was some years in securing that support which its importance justified and for which Manson so constantly and vigorously pleaded. Curiously enough it met with some opposition in the profession and only Sir Patrick's energy and the support of Mr., later Sir, Austen Chamberlain saved it from disaster; but it was 1920 before a permanent endowment and other funds were secured for the removal of the school to its present site in the University quarter of London. Soon thereafter the incorporation of the school with the London School of Hygiene was made possible by a gift of two million dollars from the Rockefeller Foundation. The last public appearance of Sir Patrick was on January 20, 1922, when students and friends presented him with a portrait and announcement was made of a medal to commemorate his work. He died on April 9, 1922, in his 78th year, having outlived most of his colleagues and contemporaries. Nevertheless many of them like Blanchard of Paris and Gorgas of Panama fame paid brilliant tributes in their writings to his work.

To the student must be left the privilege of reading this splendid biography with its effective presentation of the work of one of the world's truly great men. Only the barest outline of its contents and none of its careful analyses and discussion of scientific questions have been given here and this review may well be closed with a quotation from the book which will be of significance to every worker in this field: "By his discoveries he gave timely and very necessary point and impetus to the venerable but somewhat neglected truth that medicine, as a science, has its living roots in Biology and so led medical education back to those solid biological foundations from which it has been inclined to stray." "With none of the aids which wealth and position can bestow Manson carved out for himself a career of great distinction and created a new department of medicine." He was justly called the Father of Tropical Medicine.

Précis de Parasitologie. By Brumpt, E. Fourth Edition. 795 figures, 5 plates. 1452 pages. Masson et Cie, Paris. 1927.

The appearance of the new edition of this important and highly esteemed work is worthy of more than passing notice. The third edition which bears the date of 1922 was reviewed in the Journal for September, 1923, and it is a sign both of the remarkable advances in this field and of the ideals as well as the knowledge of the author that despite the changes recorded at that time a further revision has been undertaken so soon and has been of such thoroughly complete character. Some 250 pages and nearly 60 figures have been added to the total in the previous edition. Indeed one might well query whether the designation *Précis* is really applicable to the work in its present form. At all events the book is a mine of information well presented and accurately analyzed.

Careful reading shows that there have been changes in almost every portion of the work and even the general introductions to the various sections have been enriched along the line of recent discoveries. One of the most remarkable of these is the work done by Tate Regan on some deep sea fish. In this he demonstrated that the male has become attached to the body of the female and the union is so complete that the male forms merely an insignificant appendage on the surface of the female. The acquisition of a parasitic relation like this is of outstanding interest because of its occurrence in a group as highly organized as the fishes.

The major additions occur naturally in the group of protozoa among which the author included the spirochetes. Good use has been made of the extensive discoveries in this field during very recent years and one notices first a fine discussion of the American tick fever studied in North and South America and

Panama by various investigators. Some other less well-known and perhaps less important spirochetes are also reported in revised form. Especially noteworthy are the new figures for various species of Entamoeba. Incidentally most of these illustrations are admirable photographic reproductions of the microscopic images and would be difficult to duplicate in other texts. Similar new figures have been introduced for flagellates, *Leishmania infantum* and others. The section on flagellates has been considerably extended and modified in individual cases both by the omission of older statements invalidated by more recent discoveries and through the addition of the results of newer researches. Special attention has been called at various points to the lack of definiteness regarding present knowledge. This feature will be exceedingly useful to the student who is apt without such comment to ascribe equal certainty to all statements in a text.

Among the Sporozoa the historical review of Plasmodium has been considerable revised and new material as well as new figures introduced. Under this general heading has been included a discussion of Oroya fever and of Verruga with several new names and much new material based on recent investigations in South America. The Haemogregarines have also been worked over in rather complete fashion. On the other hand the section on Infusoria remains practically unchanged.

Among the trematodes one finds relatively few changes. The author has substituted the name *Paragonimus ringeri* of Cobbold for *P. westermanni* on the basis of the studies of the cuticular spines. He has also added items on the life history and prophylaxis of certain species both old and new in accordance with recent studies by various Japanese and other authors in the east.

Among the cestodes one finds a good many minor changes of importance but nothing very extensive except it be in the account of Hymenolepis. The new form *Diphyllobothrium minus* which he has introduced, as well as *D. parvum*, are probably according to recent researches nothing but juvenile specimens of *D. latum* as was first indicated by Zschokke. To the section on Echinococcus has been added work on the culture of the larval stages and on the alveolar type.

In the section on nematodes one finds first of all new material and figures for Ascaris and new names, the most unfamiliar of which is probably Enterobius of Leach for the well-known Oxyurias. Most students will certainly appreciate the brief synopses for the families which have been introduced in front of each genus. The section on the hookworm seems considerably enlarged and is the most up to date presentation of recent work which will be available to the student. The old genus Filaria of excessive breadth and rather indefinite character has been wisely broken up into a number of new genera that are much more natural subdivisions. The amount of new material here is noteworthy; it has added materially to the clarity of the subject and will be of great assistance to the student working in this field.

Under the ticks the author has included an extended and well written résumé of the work done by various American investigators on Rocky Mountain Spotted Fever or Tularemia. In view of very recent demonstrations concerning the spread and importance of this disease on the North American continent this addition is most timely even though the date of printing the work precluded the introduction of the most recent discoveries in this line.

More detailed consideration of various types of insects, particularly the blood sucking forms such as the Reduvidae, and of various flies which are of medical importance forms an improvement which should rightly be emphasized inasmuch as too many general works on parasitology of recent date have failed to revise adequately the material on insects. The student who regards this as a stereotyped field often owes his view to the lack of vision for which an author has been responsible. In this section Brumpt has worked over very thoroughly also the portion dealing with the mosquitoes and their various relations to disease.

Even in the section on plant parasites there have been considerable changes which are conspicuous in the nomenclature but space is wanting for a more

detailed discussion of these features. All in all the author should be highly congratulated on his success with the revision which in the language of the day is up to the minute. With such treatment no one can doubt that as prophesied in the review of the last edition the work will easily hold its place of leadership among texts in this field.

Host-Parasite Relations Between Man and His Intestinal Protozoa. By Robert Hegner, Ph.D., with 21 figures. The Century Co., New York, London, 1927, 231 pp., 21 figs.

The series of studies which Professor Hegner has been carrying on so successfully with the assistance of his students during a number of years has been brought together in the work in hand. He deals first in a comparative way with the general subject, noting the points of similarity and difference between free-living and parasitic protozoa and lists the intestinal protozoa in man and discusses the terms employed in their study. Taking up then a general account of the biology of host-parasite relations between man and his intestinal protozoa, he discusses the epidemiology of transmission, the recognized periods in natural infection, the distribution and localization of parasites within the host, the changes evoked, the adjustments affected and the therapeutics of the situation, closing this part with a section on specificity. The intestinal amoebae, the flagellates, the infusoria and the coccidia are then discussed in detail, each group in an individual chapter. A valuable section on the references to literature followed by two indexes close the work.

The book is an exhaustive treatment of the subject. The style is attractive and clear and the book will be welcomed by those who either as students of medicine or of biology are interested in this new and recently developing field. The importance of understanding such relations as are discussed in the book is at once apparent when one considers that any effective control of parasitic species must be founded on adequate and precise understanding of these varied relations. The publishers announce that this is the first volume to appear in the Century Biological Series of which Dr. Hegner is the general editor. One need hardly add that the problems discussed are among the most difficult of all relations between man and the lower organisms, and that our knowledge of the data on which such a discussion must be based is sorely inadequate.

Medical Dictionary. By George M. Gould, edited by R. J. E. Scott. Second Edition, with illustrations and one hundred seventy tables, P. Blakiston's Son & Co., Philadelphia, 1522 pp.

The new edition of this well known and admirable work comes relatively soon after the first. It includes much material which was not found in the earlier edition. Prominent among the new features is a comprehensive table of micro-organisms covering some seventy pages; the organisms are grouped under the various families and for each is given the original name, the place of occurrence, the character of the organism and its present-day classification. The bacteria and closely related organisms are fully covered and also the spirochetes. Animal micro-organisms are not handled in this way. Considering the importance which these organisms have been assigned by virtue of recent work one may hope that a new edition will give similar information concerning these groups also. Incidentally one notes that rules of spelling and nomenclature have been followed well with reference to plant organisms but have been usually disregarded in citations of animal organisms. However, a check of entries involving a considerable number of terms and species that have come into prominence very recently shows that the work is reasonably well uptodate in the special field of animal parasitology. Its comprehensiveness as well as the care with which it has been prepared make it of distinct value to the student.

Schistosomiasis vel Bilharziasis. By C. G. Kay Sharp. William Wood & Co., N. Y., 1925, 74 pp.

In a convenient volume of pocket size which is attractively printed and well written, Dr. Sharp, chief medical officer in the Education Department of Natal, has written a work that will be of value both to practitioners in the field and to teachers. He regards this as one of the great endemic diseases of the world and believes it will be eradicated in time from the ills to which the flesh is heir. From this point of view, mass treatment in schools and villages affords the hope of eradicating the disease and he emphasizes the unrivaled opportunity for educational propaganda which is offered by the school. In his view the three prophylactic means for dealing with the problem are in order of importance the following: 1. Mass treatment by antimony tartrate in schools and villages and individual cases wherever they are found. 2. Educational propaganda, to check infection and reinfection. 3. Destruction of water snails in local collections of water known to be infected.

Bolles Lee's Microtomist's Vade-Mecum, a Handbook of the Methods of Microscopic Anatomy. By J. Bronte Gatenby and E. V. Cowdry, with 9 illustrations, Ninth Edition, P. Blakiston's Son & Co., Philadelphia, 1928.

The classic volume originally prepared by Arthur Bolles Lee and now revised by Gatenby and Cowdry maintains under the new editorship its long recognized high standing. A new edition was certainly called for since work of this type changes repeatedly and the 7 years which have elapsed since the last appeared have been fruitful in the production of new methods. The present edition is considerably enlarged and has an important new section by Robert Chambers. Most of the methods are certainly up to date but some of those cited for parasites are a little old and in the event of another edition should be replaced by more recent work. Not enough has been given to make the admirable methods of Looss clear. Incidentally one of the very few misprints noted was the common omission of the final "s" in the name of that investigator. All in all, the work is a remarkable compendium and a valuable contribution for the teaching of science and one is glad to see it well maintained by the new authors.

A most exhaustive and unquestionably also important work entitled, *"A Mosquito Survey of Certain Parts of South Africa, with Special Reference to the Carriers of Malaria and Their Control,"* by Alexander Ingram, and Botha De Meillon, appeared in the publications of the South African Institute for Medical Research in October, 1927. From the point of view of public health definite knowledge of this sort is of the utmost importance and in Africa probably even more than elsewhere, since the mosquitoes are guilty not only of carrying human disease but also horse sickness.

Annaes da Faculdade de Medicina de Sao Paulo (Brazil), which might easily escape the attention of workers in medical zoology, contain numerous items of peculiar interest. In Volume II for 1927 studies on *Tripanosoma cruzi* and on blastomycoses deserve special mention.

Hunting Under the Microscope (Macmillan Co., 1928) by the late Sir Arthur E. Shipley is a most interesting series of articles chiefly on aquatic life, but the chapters on mosquitoes and on the malarial problem are so well done that they deserve at least passing mention.

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